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OXYGEN AND CARBON DIOXIDE RELATIONS OF FUSARIUM OXYSPORUM SCHLECHT. AND FUSARIUM EUMARTII CARP.[1]

JOHN P. HOLLIS[2]

(Accepted for publication March 3, 1948)

The effects of different oxygen and carbon dioxide concentrations upon fungus growth in soil have been of little interest to plant pathologists as a whole. Pure culture studies (2) have shown that species in the genus *Fusarium*, like other fungi, are tolerant of a wide range of oxygen and carbon dioxide concentrations. This fact has retarded recognition of the soil atmosphere, with its variable constitution in different soils, as playing a determinative role in the etiology of root diseases. Particularly is this true for diseases caused by species of *Fusarium*.

An attempt has been made in the present study to explore the possible effects of varied concentrations of the metabolic gases upon the growth, survival, and morphology of two species of *Fusarium* in pure culture and in soil apart from their hosts. The results are comparative in point of method and in the fact that *F. oxysporum* Schlecht. and *F. eumartii* Carp. are related taxonomically, physiologically, and pathologically. The purpose of this paper is to draw attention to certain physiological differences existing between these organisms. These differences have been recorded and evaluated in terms of survival, morphology, and relative growth rates under the various experimental conditions.

Physiological studies have been made of several important pathogens with regard to oxygen (6, 8, 12, 19, 20), carbon dioxide (2, 4, 6, 13), growth in soil (1, 7, 9, 11, 24), and forced aeration (5, 7, 10, 18).

The economic importance of diseases caused by *Ophiobolus graminis* Sacc., *Actinomyces scabies* (Thaxt.) Gussow and *Phymatotrichum omnivorum* (Shear) Duggar has provoked extensive studies which include the soil atmosphere as a possible factor controlling the extent of these fungi in particular soils. Here, as with other important soil pathogens, the role of soil moisture has been of primary interest. When air content has been considered, it is generally as a function of moisture content and soil texture.

Soil distribution studies (14, 15, 16, 17) of certain species in the genus *Fusarium* have been of aid in formulating regional patterns of their ecology in limited areas of the globe, but knowledge of distribution of these organisms in the United States has arisen mainly from reports of diseased plants and isolations from soil. Specifically we know of no reasons for the natural distribution of *F. oxysporum* Schlecht. and *F. eumartii* Carp. in the United States. It was hoped that a study of oxygen and carbon dioxide relations might yield information on this point.

[1] Published with the approval of the Director as Paper No. 438 Journal series, Nebraska Agricultural Experiment Station.

[2] Acknowledgement: The writer is grateful to Dr. R. W. Goss for helpful advice and criticism during the course of the investigation.

EXPERIMENTAL METHODS

Cultures of *Fusarium oxysporum* 293 and *F. eumartii* 257 obtained from the Department of Plant Pathology, University of Nebraska, were subjected to lowered oxygen tensions and increased carbon dioxide tensions in pure culture. Petri dishes, 100 mm. in diameter, containing measured quantities of 2 per cent potato-dextrose agar were inoculated with discs, 6 mm. in diameter, of actively growing mycelium. The reaction of the medium was standardized between pH 6.6 and 6.8. Growth measurements were recorded as the average of two diameters of the fungus colony.

Lowered oxygen tensions were obtained by using the alkaline-pyrogallol method prescribed by Buchner (3). The following equation was devised to determine quantities of alkali and acid required to establish different tensions and accounts approximately for the change in volume of chamber when alkaline pyrogallol solution is added.

$$X = \frac{(V - D)}{11}$$

where:

V = chamber volume
D = displacement of air by cultures
X = ml. 10 per cent KOH
$\frac{X}{10}$ = gm. pyrogallic acid

The initial tensions thus determined theoretically were checked manometrically and recorded in terms of mm. Hg. Gas analysis tests and amounts of nitrogen required to bring the chambers up to atmospheric pressure at the end of an experiment were carried out in some cases where the organisms ceased growth.

Limitations of this closed system method are obvious in that the supply of oxygen is gradually decreased during a test; however, it is considered to approximate certain natural conditions found in the soil more closely than the constant aeration method.

Different carbon dioxide levels were established by collecting measured quantities of the gas over water and introducing it into evacuated chambers. Exact concentrations were determined by analysis of duplicate samples and are expressed below as percentages.

Growth of the organisms in soil was studied by using mixtures of varied composition including sand, sand plus composted soil, composted soil, and field soil in glass tubes. Attempts were made to control the air content by varying composition, degree of packing, moisture content, and forced aeration. Two types of glass tubes were used. The smaller tubes, 23 cm. by 1.6 cm. internal diameter, were plugged at the lower end with glass wool and at the upper end with cotton. The tubes containing equal, weighted quantities of soil were autoclaved for 30 minutes at 15 pounds pressure, then placed upright in sterile water or dilute sucrose solution and saturated by capillary rise. To insure uniform packing and moisture-holding capacity the tubes

were dropped from a standardized height before and after saturation. Even distribution of sand particles was secured by rapidly rotating the tubes. In order to obtain the desired moisture content, it was necessary to aspirate dry air through the tubes or to place them in a drying oven until the desired weight of tube and soil was obtained. After inoculation the tubes were incubated by placing them upright in a large moist chamber which contained an air inlet plugged loosely with cotton.

The extent of growth between soil and tube was measured with a dissecting microscope and a millimeter rule and was recorded at weekly intervals. The central two-thirds of the soil cylinder was considered most representative of a given moisture level because of slight drying at the top and moisture accumulation at the bottom of the tube. Isolations were made from the center of the soil cortex.

Large tubes of 2.2 cm. internal diameter and 25.4 cm. in length were used in forced aeration experiments. These tubes were held upright with sterile air entering at the lower end.

All experiments, with one exception to be noted below, were carried out under controlled laboratory temperatures of 25° ± 1.5° C.

GROWTH, SURVIVAL AND MORPHOLOGY UNDER LOW OXYGEN TENSIONS

Preliminary tests showed that the growth ratio between the two organisms in air approximated 2 to 1, with *Fusarium oxysporum* making the more rapid growth. Figure 1 shows how this ratio was modified to 4 to 1 when both organisms were placed in a large 6.5-liter desiccator from which all oxygen was removed. Sufficient oxygen was dissolved in the medium and in water used in maintaining high humidity to permit some growth. Four cultures of *F. eumartii* were dead when restored to air. Restoration growth was based on 2 cultures.

Further tests with initial oxygen tensions of 14.6 (two tests) and 51.1 mm./Hg. were conducted with the same desiccator. The lower tension yielded results similar to those given above even when the time of exposure was shortened to 6 days. The normal growth ratio of 2 to 1 was maintained under a time-tension relation of 3.25 days and tension of 51.1 mm./Hg. Growth was progressing when the cultures were removed to air. The presence of oxygen in chambers where growth had ceased could not be detected by the methods described above.

Subsequent tests with Hempel gas jars and a series of different oxygen tensions established simultaneously, showed that the growth rate of *Fusarium oxysporum* after removal to air was inversely proportional to the oxygen tension imposed at the beginning of the experiment. The explanation of this phenomenon as it occurs in invertebrates has been explained by Von Brand (25). He attributes it to an oxygen debt incurred by an organism during the period of stay in oxygen-poor surroundings. This results in an oxygen intake greater than usual after restoration to a normal oxygen tension. The surplus oxygen is used to eliminate non-oxidized or partially

oxidized products formed during the preceding period of partial asphyxiation.

Previous work by Goss[3] showed that *Fusarium eumartii* could not survive prolonged exposure to anaerobic conditions in pure culture. Accord-

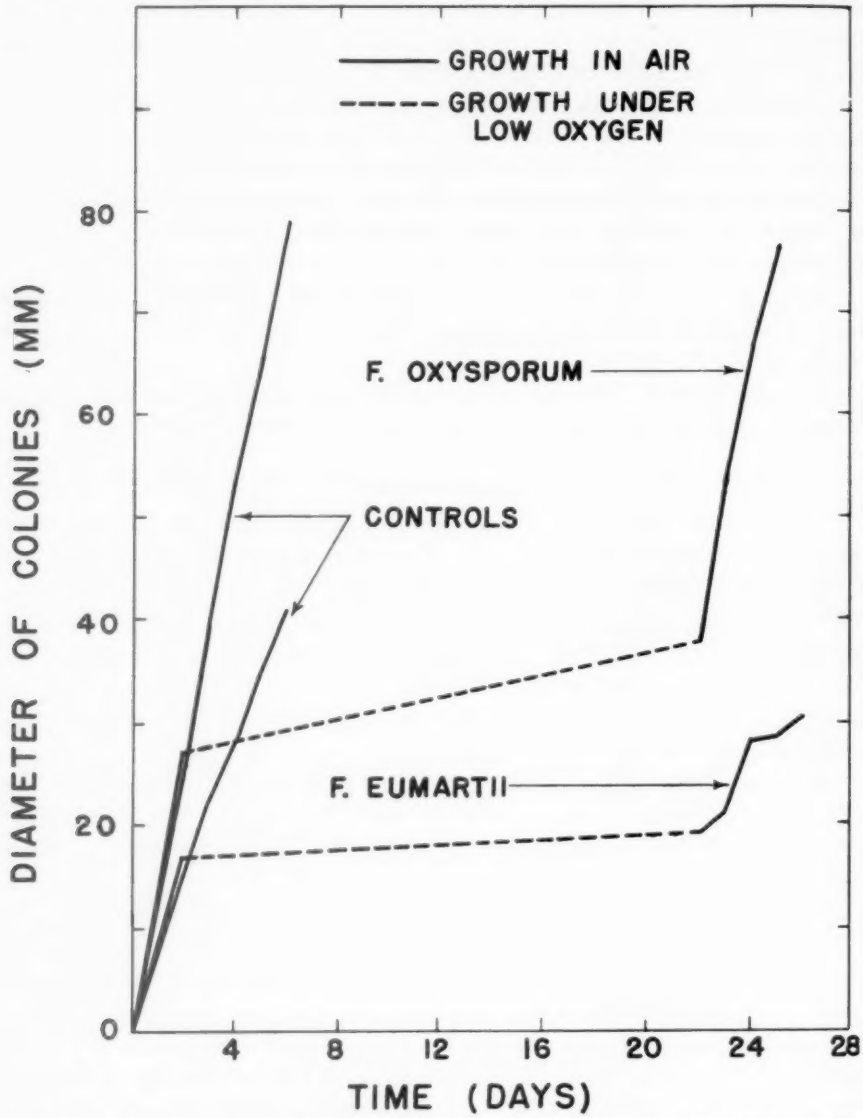

FIG. 1. Growth of the organisms under an initial oxygen tension of 0 mm./Hg. over a 20-day period compared with growth of control cultures.

ingly, 48-hour cultures were sealed in test tubes with paraffin and cork. Volatile substances were removed from the paraffin by holding it slightly above the melting point for one week. Control tubes were plugged with

[3] Goss, R. W. Unpublished work. Nebr. Agr. Exp. Sta.

cotton and covered with aluminum foil. All tubes were incubated at 25° C. for 13 weeks. Platings showed that all cultures of *F. oxysporum* were viable, whereas all cultures of *F. eumartii* were non-viable. The experiment was repeated for a period of 3 weeks and yielded identical results.

Morphological changes induced by hyphal tips of *Fusarium oxysporum* 293 growing under low oxygen tension are shown in figure 2, part A. Normal hyphae are provided for comparison. Both drawings are projection copies of photomicrographs. The first culture was placed under an initial oxygen tension of 73 mm./Hg. and held for 8 days. Similar growth was expressed under a range of oxygen tension-time relations, thus colonies started at initial tensions of 12, 31, and 73 mm./Hg. and held for 3, 4, and 8 days exhibited essentially the same morphologic response. Negative results at an extremely low oxygen tension were helpful in showing the nature of this response. Cultures placed in a chamber subjected to prolonged evacuation at an atmosphere pressure of 0.0003 mm./Hg. and retained there for 48 hours had a different morphology. Hyphal branching was repressed. The branches were short, averaging less than 10 μ in length.

Four types of laboratory media were used in these experiments in addition to potato-dextrose agar. The morphologic reaction was similar on all media tested.

Interesting observations were made of the recovery phenomenon in *Fusarium oxysporum* after removal of cultures to air. Cultures, seventy-two hours old, of *F. oxysporum* were subjected to an initial oxygen tension of 43.8 mm./Hg. and held for 3 days in a Hempel gas jar under a gradually decreasing tension. Nitrogen was added to make up the bulk of the atmosphere in the chamber. Microscopic examination showed that hyphal tips began to rupture and extrude their protoplasmic contents 2 hours after the colonies were restored to air. Rupturing of tips became more frequent between 2 and 3 hours after restoration. Normal hyphae began to elongate at the expense of their neighbors and growth along the entire margin was soon resumed. No rupturing of hyphal tips was observed after 15 hours. Morphologic changes of this character could not be induced in *F. eumartii*. All cultures had normal colony margins.

It was thought this morphologic response in *Fusarium oxysporum* 293 might be common to other species in the section Elegans. Accordingly, 5 additional isolates[4] falling into this section and 12 isolates[5] representing forms described by Snyder and Hansen (21, 22, 23) were tested. All results were negative. *F. oxysporum* 293 was the only isolate, of any tested, which gave a positive morphologic response to limiting oxygen tensions. The two additional Nebraska isolates of *F. oxysporum* have been cultured for more than 3 years, while *F. oxysporum* 293 has been cultured for more

[4] Two isolates of *Fusarium oxysporum cubense* from C. H. Meredith, one isolate of *F. lini* from J. J. Christensen, two additional Nebraska isolates of *F. oxysporum*.

[5] Forms of all species described, with the exception of *Fusarium nivale* (Fr.) Ces., received from W. C. Snyder. These included three forms each of *F. oxysporum* and *F. solani*.

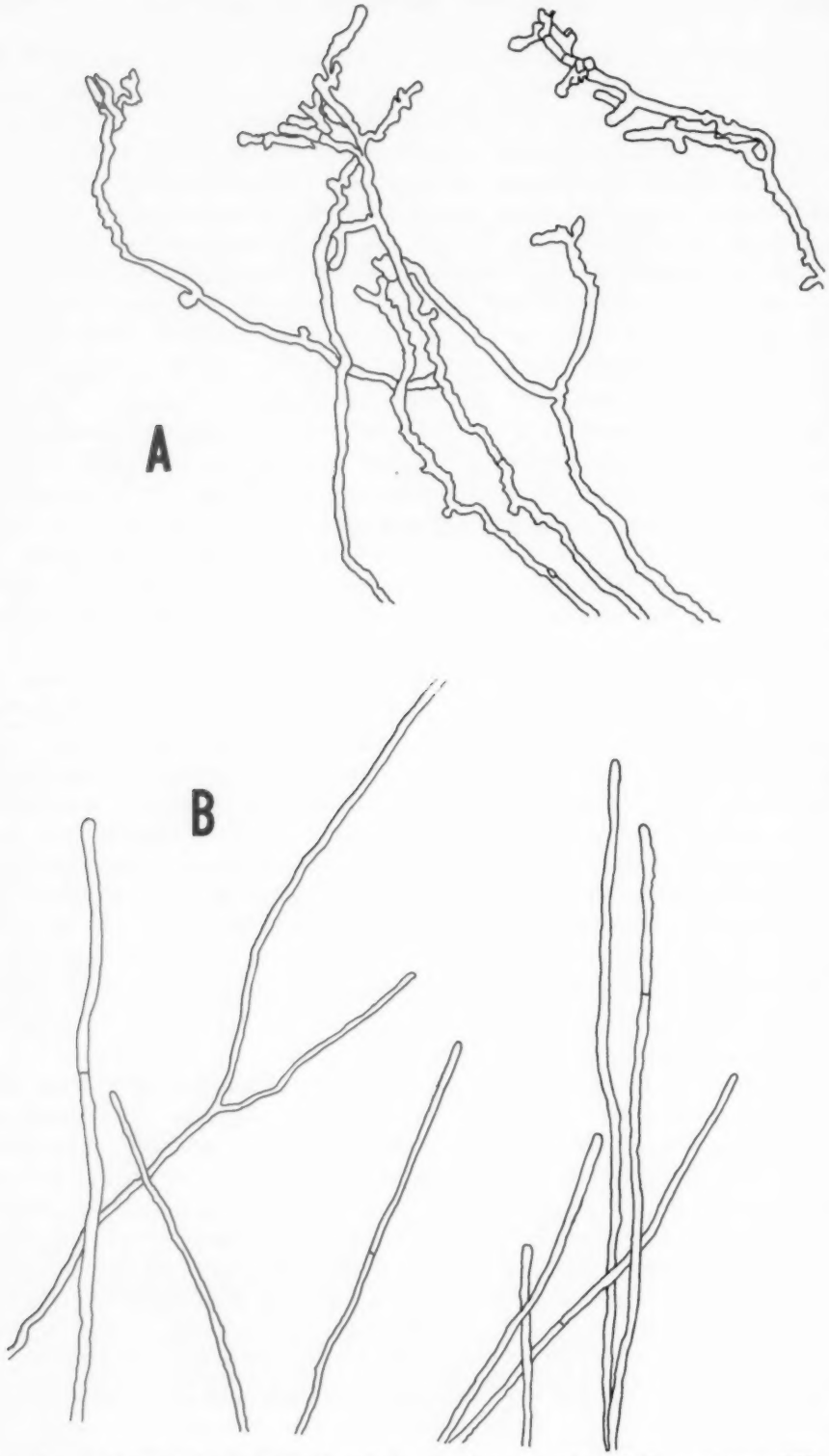

FIG. 2. Drawings of photomicrographs of colony margins of *F. oxysporum* 293 at 375 diameters. A. Morphologic reaction to an initial oxygen tension of 73 mm./Hg. B. Normal hyphae in free air.

than 10 years and the morphologic reaction peculiar to it has been observed for more than 6 years.

An experiment on growth-temperature relations at limiting oxygen tensions and temperatures of 10°, 15°, 20°, 25°, and 30° C. showed no shift in the optimum temperature for growth of *Fusarium oxysporum* on an artificial medium. Similarly, no measurable growth was recorded for *F. eumartii.*

The present data show that *Fusarium oxysporum* 293 ceases growth at an oxygen level well below 1 per cent and survives, while *F. eumartii* ceases growth at a higher oxygen level and dies under the anaerobic conditions that follow. While *F. oxysporum* 293 is utilizing oxygen, beginning at a level ranging from 3 to 10 per cent down to less than 1 per cent at the end of an experiment, definite morphological changes in the character of hyphal growth take place. These changes do not occur in *F. eumartii* when this organism is grown under the same conditions.

CARBON DIOXIDE RELATIONS

Preliminary experiments showed that these organisms were tolerant to a wide range of carbon dioxide concentrations. Accordingly, 8 desired concentrations ranging from 3.5 to 75.3 per cent were established. The general procedure has been described above.

The experimental unit was a Hempel gas jar containing 3 cultures of each organism. Four chambers were available making it possible to run 4 concentrations in each test. In order to maintain the desired carbon dioxide level and to measure daily growth, the concentrations were renewed at 24-hour intervals. Each test was repeated twice at nearly equal concentrations providing a total of 9 cultures.

Control cultures were held for each test as indicated. It must be stated that concentrations were randomized, *i.e.*, no particular sequence of carbon dioxide concentrations was employed in any one test.

Figure 3 represents the data from 6 successive experiments. The results show that both organisms made good, measurable growth over a wide range of carbon dioxide concentrations. Over the 4-day period the highest concentrations reduced growth of *Fusarium oxysporum* to less than one-fourth and in *F. eumartii* to about one-third of the normal amount, indicating that *F. oxysporum* is less tolerant to high concentrations of carbon dioxide than *F. eumartii.*

Oxygen was suspected as a limiting factor at the highest carbon dioxide concentrations but calculation showed that the oxygen level was about 6.17 per cent when the carbon dioxide concentration was 75.3 per cent. Previous experiments showed that this is not a limiting oxygen value when the mixture is renewed daily in a chamber of this size. A generalized conclusion is that growth of neither organism can be much affected by the carbon dioxide in the soil atmosphere when the concentration is below 48 per cent. At this point the growth of *Fusarium oxysporum* was reduced by half while that of

F. eumartii was reduced by only one-third. Figure 3 shows stimulation in the growth of *F. oxysporum* at about 3.5 per cent carbon dioxide. Similar responses have been noted by Lundegårdh (10) for *F. culmorum* and *Gibberella saubinetii* in soil.

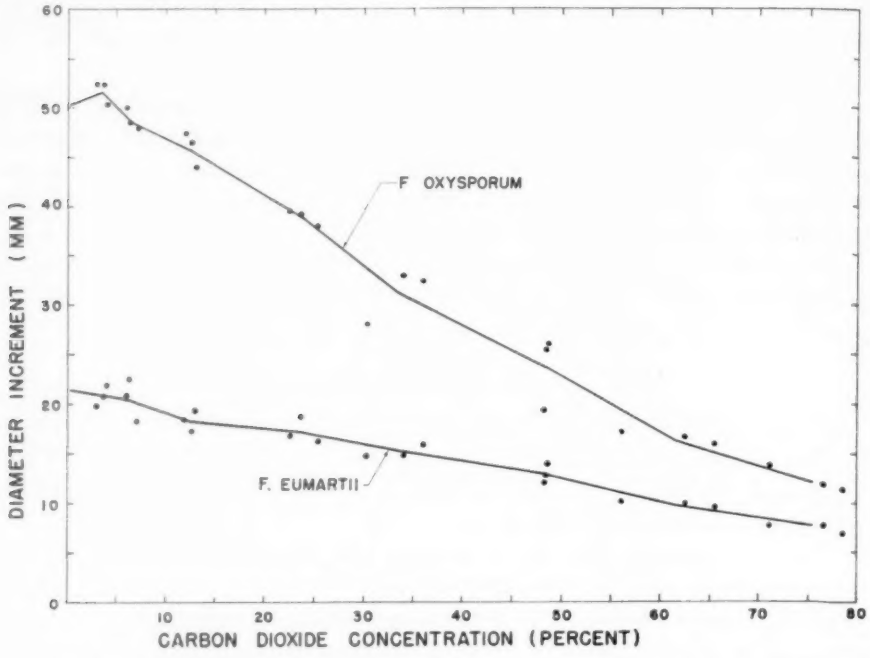

FIG. 3. Comparative growth of the organisms over a 4-day period in pure culture at 25 different concentrations of carbon dioxide.

GROWTH IN SOIL

Preliminary experiments indicated that growth of the organism in soil is stimulated by air pockets. Visible growth in the tubes was especially profuse in open areas between soil and tube. This point is well illustrated by the results of an experiment conducted with sand of three particle sizes. The sand was washed in distilled water and passed through standardized screens to yield particles of the following size ranges: coarse, 1–0.5 mm.; medium, 0.5–0.25 mm.; and fine, 0.25–0.1 mm. Moisture-holding capacity of each size series was determined by saturating 1 tube from each series, draining for 2 hours and reweighing. The M.H.C. of coarse sand was 24.8 per cent; medium sand, 32.0 per cent; and fine sand, 34.3 per cent.

The tubes were saturated with a 5 per cent sterile sucrose solution and inoculated with a 0.5-ml. aqueous spore suspension. To prevent drying of the sand and to limit passage of air into the tubes, parafilm paper was placed over the ends and held securely with rubber bands. Three tubes were used for each organism and each sand particle size. Growth between sand and tube was recorded after 10 to 20 days. No significant differences could be

seen between growth of the two organisms but total growth in coarse sand during the second 10-day period was 15.3 cm.; in medium sand, 1.7 cm.; and in fine sand, 0.0 cm.

An experiment was made in which the independent variable was moisture content. A mixture of 60 per cent medium sand and 40 per cent coarse compost was adjusted to moisture levels in the tubes of 60, 80, and 100 per cent of M.H.C. Agar discs (1.5 cm.) from actively growing colonies were used for inoculations.

The results over a 4-week period were exceedingly variable. No differences in growth could be detected at different moisture levels but the growth ratio between the two organisms at all moisture levels approximated that

TABLE 1.—*Depth of isolations made from center of soil cylinder at conclusion of experiment with Fusarium oxysporum and F. eumartii growing in a sand-composted soil mixture*

Tube number	Organism	Percentage of M.H.C.	Viability of isolations from different depths		
			One-half visible growth	Visible growth	1 cm. beyond visible growth
No.	*Type*	*Per cent*	*Viability*	*Viability*	*Viability*
1	*F. oxysporum*	100	+	+	+
2	*F. oxysporum*	100	+	+	−
5	*F. eumartii*	100	+	+	−
6	*F. eumartii*	100	+	+	−
9	*F. oxysporum*	80	+	+	−
12	*F. oxysporum*	80	+	+	+
13	*F. eumartii*	80	+	+	+
14	*F. eumartii*	80	+	+	+
17	*F. oxysporum*	60	+	−	−
18	*F. oxysporum*	60	+	+	+
21	*F. eumartii*	60	+	+	+
22	*F. eumartii*	60	+	+	+

obtained in pure culture. The average weekly increment of *Fusarium oxysporum* was approximately 20 mm. while that of *F. eumartii* was about 12 mm. Table 1 shows the results of isolations made on Burkholder's medium. Greatest penetration of the soil cortex by both organisms was made at moisture levels of 60 and 80 per cent of M.H.C. Bacterial contamination was present in tubes held at 100 per cent of M.H.C. A similar experiment was conducted using large tubes and forced aeration. Moisture levels in uncomposted top soil were 40, 60, and 100 per cent M.H.C., which was determined as 53 per cent of oven-dry weight. The tubes were plugged with cotton at both ends and inoculated with a 1-ml. spore suspension and a 20-mm. agar disc inverted on top of the soil columns and covered with moist sterile sand. The aeration train for each tube consisted of a concentrated NaOH trap and a water-bottle used for regulating the rate of air flow with a screw clamp. This arrangement was later used to determine CO_2 evolution by replacing water with alkali.

Isolations were made from 6 levels in each tube after 24 days onto acidulated and neutral Burkholder's medium (Table 2). Again, the best penetration was made at medium and low moisture levels. Bacterial contamination was present again in tubes held at 100 per cent of M.H.C. No antagonism between the fungi and bacterial contaminants could be detected in pure culture.

Activity of the organisms in composted soil with a M.H.C. of 37.5 per cent was studied by determining carbon dioxide evolution over an 8-day period. Moisture content of the soil was adjusted to 48 per cent of M.H.C. The tubes were sterilized for 1 hour at 15 lb. pressure on three alternate days over a 6-day period. Six of the 8 tubes were inoculated with a 1-ml. spore suspension and the remaining 2 were held as controls. After 7 days, 5 and 15 ml. of sterile water were added to the first two tubes of each series,

TABLE 2.—*Depth of isolations made from center of soil cylinder at conclusion of experiment with Fusarium oxysporum and F. eumartii growing in uncomposted top soil under forced aeration*

Tube number	Organism	Percentage of M.H.C.	Viability of isolations					
			Depth (cm.)					
			2	4	6	8	12	15
No.	*Type*	*Per cent*	*Viability*					
1	*F. oxysporum*	100	+	+	−	−	−	−
2	*F. eumartii*	100	+	−	−	−	−	−
3	Control	100	−	−	−	−	−	−
4	*F. oxysporum*	60	+	+	+	+	+	+
5	*F. eumartii*	60	+	+	+	−	−	−
6	Control	60	−	−	−	−	−	−
7	*F. oxysporum*	40	+	+	+	+	+	−
8	Control	40	−	−	−	−	−	−

respectively, to determine if this would result in an increase in activity over the eighth day. The remaining tubes were weighed to determine water loss.

The initial rise (Fig. 4) was probably due to the stimulus of inoculum and CO_2 trapped in the system at the start of the experiment. Weighing of unwatered tubes showed a water loss ranging from 2 to 5 gm. This represents an average loss of about 13 per cent. This loss, coupled with the low initial moisture level, accounts for the low level of activity over the course of the experiment. The increase in CO_2 evolution over the last day again can be attributed to two factors: CO_2 trapped in the system and increased activity of the organisms.

Growth of the organisms between a glass surface and sand of different particle size showed that it is possible to control the rate of growth with size of sand particle. The results do not show whether this is primarily due to air content or soil space. High nutrient level was eliminated as a factor because greatest growth occurred in the presence of lowest nutrient supply. Tolerance of the organisms to lowered oxygen tensions in pure

culture coupled with the results discussed above, suggest that the size of soil spaces may be an important factor determining growth in soil. Meredith (11) found that *Fusarium oxysporum cubense* made an average daily growth on the surface of greenhouse compost in long glass tubes of 0.47 cm. per day, whereas the daily average on clay was only 0.3 cm. Here the effects of

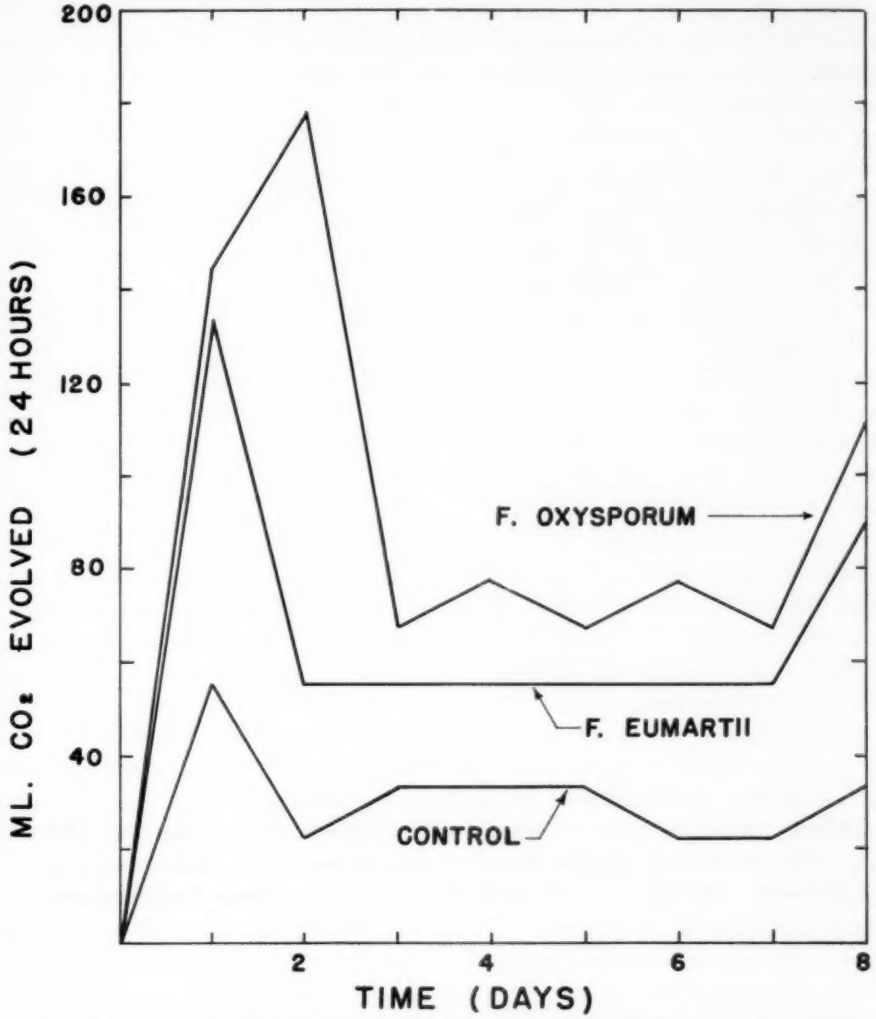

FIG. 4. Carbon dioxide evolution by the organisms in greenhouse compost at approximately 48 per cent of M.H.C. (M.H.C. 37.5 per cent of dry weight).

air content must have been negligible since the tubes were only half filled with soil. Nutrient level and soil space may have determined primarily the rates of growth.

Greater penetration of both unaerated and aerated soil at lower moisture levels by *Fusarium oxysporum* and *F. eumartii* is more difficult to explain. Higher moisture levels may have limited growth indirectly by cutting down

soil space as well as air content. This could have occurred with swelling of the organic colloids and their cementing together with bacterial gum. Meredith (11) found that sandy soils permitted greater penetration by *F. oxysporum cubense* than loamy soils. Both soil space and air content may have been important in bringing about this difference.

The organisms used in this study are capable of independent growth in the soil similar to that demonstrated by Blair (1) for *Rhizoctonia solani* Kühn. Growth proceeds primarily through spaces in the soil and along solid surfaces.

DISCUSSION

This work has emphasized the tolerance of both organisms to oxygen and carbon dioxide. Survival under anaerobic conditions and greater relative growth under low oxygen tensions indicate that *Fusarium oxysporum* is a more versatile organism than *F. eumartii*.

It is impossible to draw conclusions from the present data relative to allied species or forms in the sections Elegans and Martiella. It was seen, for example, that a positive morphologic response to lowered oxygen tensions by *Fusarium oxysporum* 293 was absent in 2 other isolates of *F. oxysporum* and in 17 other species and forms of the genus tested. Different isolates of the same species might also differ from one another in their ability to grow under low oxygen tensions.

Nowhere have differences between species and forms of the genus been expressed as in the work of Reinking and Manns (16, 17) and Reinking (14, 15). The first study (16) located in Honduras and Guatemala included some 15 soil types comprised mostly of sandy-clay and loam mixtures. It was reported that 24 different species, varieties, and morphologic forms of the genus *Fusarium* were isolated from surface soil in the areas sampled. The greatest number of types were from the section Elegans and 3 of these types were found in nearly all surface soils. It is interesting that *F. solani* var. *eumartii* was found to occur in only a few soil types, but was found commonly in isolations from all depths examined. Indeed, they classified this organism as a soil inhabitant on the basis of isolations from depths of the soil below a half inch. There is little agreement between their findings and those presented in this paper. There is, however, agreement between relative frequency of forms of *F. oxysporum* and the more widespread occurrence of oxysporum wilts as compared with the more sporadic distribution of Martiella species and their corresponding root rots. It is generally recognized that oxysporum wilt of potato is more widespread than eumartii wilt.

Reinking and Manns (14, 15) further found that the majority of *Fusarium* spp. obtained were isolated in greater relative numbers from light acid soils. Concerning the distribution of Panama disease, Wardlaw (26) states in part, "The collective experience, practical and scientific, in Central America is that hydrogen ion concentration is the master factor determining

the severity of Panama disease, followed by texture." Recently Wardlaw (27) reported results of an interesting experiment carried out in Honduras. Land which had been removed from cultivation, because of the Panama disease (*Fusarium oxysporum cubense*), was divided into sections and flooded for periods ranging from 6 to 18 months. The fungus presumably died of oxygen starvation, since control of the disease was secured for as long as 6 years after treatment although isolated plants were found to be infected after 10 to 18 months. While the present results are at variance with this report, it must be remembered that we are dealing with related organisms which were tested in two different substrates. The author's own data warrant the speculation that antibiosis may have been important in eliminating *F. oxysporum cubense* from this flooded soil and in line with the phenomenon of recovery above, that frequent short periods of flooding might be more effective in promoting antagonism to the fungus than one prolonged period. Wardlaw further states, "It is considered improbable that the method of flood-following will ever be successful in acid soils (in which plants succumb readily and in which the fungus may spread with great rapidity), but the system is being extended to other areas considered to be suitable." The present results suggest that soil space as controlled by texture may be a more important factor in the distribution of these organisms than soil air content. More work is needed to separate the effects of texture and air content upon fungus growth in soil.

We cannot account for differences in ecology of the organisms on the basis of their reaction to oxygen since both can grow at extremely low tensions, but these results indicate that *Fusarium oxysporum* is better equipped for survival as a saprophyte than *F. eumartii* and that the latter organism might tend to die out more readily in a water-logged soil.

SUMMARY

1. The effects of different oxygen and carbon dioxide concentrations upon growth and morphology of *Fusarium oxysporum* and *F. eumartii* were studied in pure culture. Growth in sand and various mixtures of sand and compost was studied in relation to moisture content, texture, and forced aeration.

2. *Fusarium oxysporum* had greater tolerance to lowered oxygen tensions than *F. eumartii*.

3. Sealed cultures of *Fusarium oxysporum* were viable after 13 weeks, whereas those of *F. eumartii* were non-viable after 21 days.

4. Both organisms had considerable tolerance to high carbon dioxide concentrations.

5. *Fusarium oxysporum* 293 exhibited a definite morphologic reaction to lowered oxygen tensions in pure culture.

6. This morphologic reaction did not occur in any of 19 additional isolates of the genus *Fusarium* including 2 isolates of *F. oxysporum*.

7. In an unaerated sand-composted soil mixture nearly equal penetration was obtained at all moisture levels, with the growth ratio approximating that obtained in pure culture.

8. In both unaerated and aerated soil, greater penetration was obtained at moisture levels below saturation.

9. Under forced aeration in composted soil the organisms were active at moisture levels below 48 per cent of moisture-holding capacity.

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YELLOWS AND NECROTIC RING SPOT OF SOUR CHERRIES IN ONTARIO[1]—DISTRIBUTION AND SPREAD

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Under one name or another, yellows of sour cherries has been known in Ontario and elsewhere for many years. The disease had become widely distributed in cherry growing districts before 1939, when its infectious nature was demonstrated by Keitt and Clayton (6) in Wisconsin. Moreover, the absence of definite symptoms in late summer makes it difficult to propagate yellows-free nursery stock, unless great care is exercised in the selection of parent trees. There is, therefore, good reason to believe that most of the present plantings of sour cherries have been more or less contaminated with yellows from the start. On the other hand, the annual recurrence of symptoms in late June (7), though varying greatly in intensity from season to season, makes it possible to follow with comparative ease the progress of the disease in an orchard over a period of years.

In Ontario, the first known cases of necrotic ring spot were observed on Montmorency cherry in the spring of 1939 (3), and the virus nature of the disease was demonstrated soon afterwards. At about the same time, necrotic ring spot, or a very similar disease, was discovered independently in various parts of North America (4, 5, 10) on sour cherry or other hosts. The symptoms of the disease have been considered in detail elsewhere (1, 3, 4, 5) and need not be discussed here, except to mention the apparent recovery of affected trees soon after infection. Furthermore, indexing experiments (1) have shown that many apparently healthy sour cherry trees carry necrotic ring spot in latent form and, on the other hand, that a few do not. It would thus appear that this disease, even more than yellows, is frequently and unwittingly propagated in the nursery and set out in the orchard. Because of the tendency to recovery and the consequent irregular occurrence of diagnostic symptoms after the first year of infection, there is considerable uncertainty concerning both the actual incidence of necrotic ring spot in a given orchard and the rates and conditions of spread, facts which could be determined with any degree of accuracy only by the wellnigh impossible task of indexing every apparently healthy cherry tree in the orchards under consideration. The present paper is an attempt to come to some conclusion on these points by means of an analysis of data obtained from orchard surveys.

MATERIAL

Since 1941, a number of Montmorency cherry orchards, one for yellows alone, two for necrotic ring spot alone, and three for both diseases, have

[1] Contribution No. 919 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Canada.

been under observation for three or more successive years. Each orchard was charted to show the known distribution of each disease at the time of the first survey and the location of the new cases appearing in each succeeding year. In addition, the distribution of yellows was mapped in five more orchards for the first time in 1945.

INCIDENCE

The incidence of each disease in the several orchards in the first survey and in 1945, as well as the annual increases were calculated as percentages of the number of trees per orchard (Tables 1 and 2). Since, for reasons already mentioned, the significance of the necrotic ring spot data may be open to question, it was considered advisable to submit alternative inter-

TABLE 1.—*Percentages of Montmorency cherry trees in various orchards infected with yellows, at the first survey, and becoming infected each year thereafter*

Orchard No.	Age in 1945	No. trees	Initial incidence	New infections				Mean increase per annum	Incidence in 1945
				1942	1943	1944	1945		
1	15	432	22.0[a]	3.2	11.8	9.7	20.8	11.4 ± 3.6	67.5
2	8	288	3.5	—[b]	—	1.4	6.6	4.0 ± 2.6	11.5
3	12	72	2.8	5.5	4.2	2.8	12.5	6.3 ± 2.2	27.8
4	20	138	9.4	2.2	4.7	2.3	2.4	2.9 ± 0.6	21.0
Mean (orchards 1–4)			9.4 ± 4.5	3.6 ± 1.0	6.9 ± 2.5	4.1 ± 1.9	10.6 ± 4.0	6.4 ± 1.5[c]	31.9 ± 12.3
5	7	66	—	—	—	—	—	—	45.5
6	10	180	—	—	—	—	—	—	27.7
7	3	388	—	—	—	—	—	—	18.8
8	3	224	—	—	—	—	—	—	26.8
9	18	84	—	—	—	—	—	—	100.0
Mean incidence in 1945 (orchards 1–9)									38.5 ± 9.5

[a] In tables 1 and 2 initial incidence is for the year immediately preceding the first record of new infections, *e.g.*, in orchard 2, the incidence of yellows was 3.5 in 1943.
[b] —, No records taken.
[c] Mean of all readings of new infections.

pretations, each serving as a check on the other. The first interpretation, based on the assumption that new cases arose in the year in which symptoms were first detected or suspected, gives what might be termed "apparent annual spread." If, however, the severity of the observed symptoms is taken into account, as befits the known characteristics of necrotic ring spot, the "probable annual spread" can be computed. For this purpose, the few trees in which infection was only suspected were regarded as "healthy," while those exhibiting only mild symptoms were classified as having been affected before the surveys began and those with severe or moderately severe symptoms as representing the new infections. Since neither interpretation solves the problem of the health status of the trees in which no symptoms were observed, only the "minimum incidence" of necrotic ring spot in each orchard can be known. The assigned values of minimum in-

TABLE 2.—*Percentages of Montmorency cherry trees in various orchards, known to be infected with necrotic ring spot at the first survey, and becoming infected each year thereafter*

Orchard No.	Initial incidence (minimum)	New infections				Mean increase per annum	Incidence in 1945
		1942	1943	1944	1945		
Apparent incidence and spread[a]							
1	42.8	—	—	11.3	5.8	8.6 ± 2.8	60.0[c]
2	42.0	—	—	7.0	13.5	10.3 ± 3.2	62.5
3	4.2	20.8	25.0	22.2	0.0	17.0 ± 5.7	72.2
5	27.3	—	18.2	9.1	3.0	10.1 ± 4.4	57.6
6	17.8	—	—	12.2	9.4	10.8 ± 1.4	39.4
Mean	26.8 ± 7.3	20.8	21.6 ± 3.4	12.4 ± 2.8	6.3 ± 2.4	12.1 ± 2.1	58.3[c] ± 5.4

Orchard No.	Initial incidence (minimum)	New infections					Mean increase per annum	Incidence in 1945
		1941	1942	1943	1944	1945		
Probable incidence and spread[b]								
1	34.5	—	—	14.6	5.8	1.8	7.4 ± 3.8	56.7[c]
2	24.6	—	—	19.5	5.2	6.6	10.4 ± 4.6	55.9
3	29.3	5.5	18.0	13.9	5.5	0.0	8.6 ± 3.2	72.2
5	16.7	—	24.2	6.1	6.1	3.0	9.9 ± 4.8	56.1
6	26.7	—	—	0.0	3.3	6.1	3.1 ± 1.8	36.1
Mean	26.4 ± 2.9	5.5	21.1 ± 3.1	10.8 ± 3.4	5.2 ± .5	3.5 ± 1.3	8.1 ± 1.6	55.4[c] ± 5.7

[a] Infections date from year in which any symptoms were first observed.

[b] Infections date from year in which severe symptoms occurred; trees with mild symptoms classified under initial incidence.

[c] Calculations of apparent incidence and spread include suspected cases; those of probable incidence and spread do not.

cidence for any year would of course vary according to the interpretation of the data. On the other hand, comparison of the two points of view is of value in considering the rate and manner of spread.

Since both diseases are infectious, it was thought that the amount of disease present in an orchard at any time would have an important bearing on rate of spread. Accordingly the incidence of each disease in indi-

TABLE 3.—*Coefficients of correlation between the percentage of diseased trees in an orchard in any one year and the percentage of new infections appearing the next year*

Disease	A[a]		B[b]	
Cherry yellows	0.729	(1 per cent)[c]	0.815	(< 1 per cent)
Necrotic ring spot				
(apparent spread)	−0.656	(2 per cent)	−0.331	(25–30 per cent)
(probable spread)	−0.503	(3 per cent)	−0.276	(25–30 per cent)

[a] Correlations using percentage increases calculated in terms of the number of trees in the orchards (See tables 1 and 2).

[b] Correlations using percentage increases calculated in terms of the number of "healthy" trees available for infections. The percentages of previously diseased trees were the same for both columns.

[c] Levels of significance.

vidual orchards in each year was correlated with the corresponding amount of spread in the next year (Table 3). The first column of coefficients was calculated from the information given in tables 1 and 2. However, the fact that the percentage of trees to which the disease can spread in a given orchard decreases automatically as the incidence rises, tends to give those correlations a negative bias. To correct this tendency, incidence was also correlated, as shown in the last column of table 3, with percentage increases calculated in terms of the number of trees remaining "healthy" the previous year (Table 4).

With yellows, there was a wide variation both in incidence from orchard to orchard and in the rates of spread in different orchards and seasons (Tables 1 and 4). It might be expected that older orchards would be more severely attacked than younger ones, but the 1945 incidence of yellows was not significantly correlated with age of orchard. Also, the rates at which the disease increased were independent of the age factor. Similar results were obtained by Keitt and Clayton (7), who reported an average increase of 3.5 per cent per annum in five orchards during the year 1936 to 1940. It is scarcely necessary to point out that the current percentage of affected trees in an orchard depends not only on the length of time to which the trees have been exposed to infection, but also on the number and location of diseased trees set out with the orchard, on the presence or absence of other sources of inoculum, on the nature and prevalence of the disseminating agent or agents, and on other factors which may influence the annual rate of spread. The highly significant correlation between the amount of spread and the amount of disease previously present (Table 3) indicates that the probability of the spread of yellows from tree to tree increases with the number of sources of infection and confirms by inference the view that trees which do not exhibit symptoms may be presumed yellows-free. On the other hand, since the average annual rates of increase per orchard were not proportional to the initial observed incidence (Tables 1 and 4), the rate of spread of yellows in an individual orchard was evidently modified by some other factors of local significance.

The successive patterns of distribution of yellows-infected trees in each block (Fig. 1) provide some evidence that the relative position of sources of infection in an orchard can also play an important part in the later history of the disease in that orchard. For example, the diseased trees, at the time of the first survey in 1941, were massed along the north side and towards the west end of orchard 1 (Fig. 1). In orchard 2, a younger block adjoining the south side of orchard 1, the first diseased trees were mostly scattered singly and at random. Here the average increase of yellows per annum was much less than in orchard 1 (Table 1). The later surveys showed that new infections in both blocks tended to arise with greater frequency to the east and south of potential sources than to the west and north. There also appeared to be some easterly and southerly drift of infection from the older orchard to the adjacent half of the

TABLE 4.—*Percentages of previously "healthy" trees becoming diseased in the year indicated*

Orchard No.	Cherry yellows					Necrotic ring spot										
	Increase in				Av. increase per annum	Apparent increase in[a]				Av. increase per annum	Probable increase in[b]					Av. increase per annum
	1942	1943	1944	1945		1942	1943	1944	1945		1941	1942	1943	1944	1945	
1	4.2	15.8	15.4	39.1	18.6 ± 7.4	—	—	19.8	12.6	16.2 ± 3.6	—	—	22.2	11.4	4.1	12.6 ± 5.3
2	—	—	1.5	6.9	4.2 ± 2.7	—	—	12.0	26.5	19.3 ± 7.3	—	—	25.8	9.3	13.0	16.0 ± 5.0
3	5.7	4.5	3.2	14.8	7.1 ± 2.6	21.8	33.3	44.4	0.0	24.9 ± 9.5	7.8	26.6	29.4	16.7	0.0	16.1 ± 5.6
4	2.4	4.9	2.8	2.9	3.3 ± .6	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	25.0	16.6	6.7	16.1 ± 5.3	—	29.1	10.3	11.4	6.5	14.3 ± 5.0
6	—	—	—	—	—	—	—	14.9	13.5	14.2 ± .7	—	—	0.0	4.5	8.7	4.4 ± 2.5
Av.	4.1 ± 1.0	8.4 ± 3.7	5.7 ± 3.2	15.9 ± 8.1	8.9 ± 2.7	21.8	29.2 ± 4.2	21.5 ± 5.9	11.9 ± 4.2	19.0 ± 3.3	7.8	27.8 ± 1.3	17.5 ± 5.4	10.7 ± 2.0	6.5 ± 2.2	13.2 ± 2.3

[a] New infections date from year in which any symptoms were first observed.
[b] New infections date from year in which severe symptoms occurred; trees with mild symptoms classified under initial incidence. (See table 2.)

younger one. In orchard 3, in which a relatively rapid increase occurred, the first new infections appeared to the north of one of the original sources (Fig. 1). These possibly came from an adjoining sweet cherry orchard

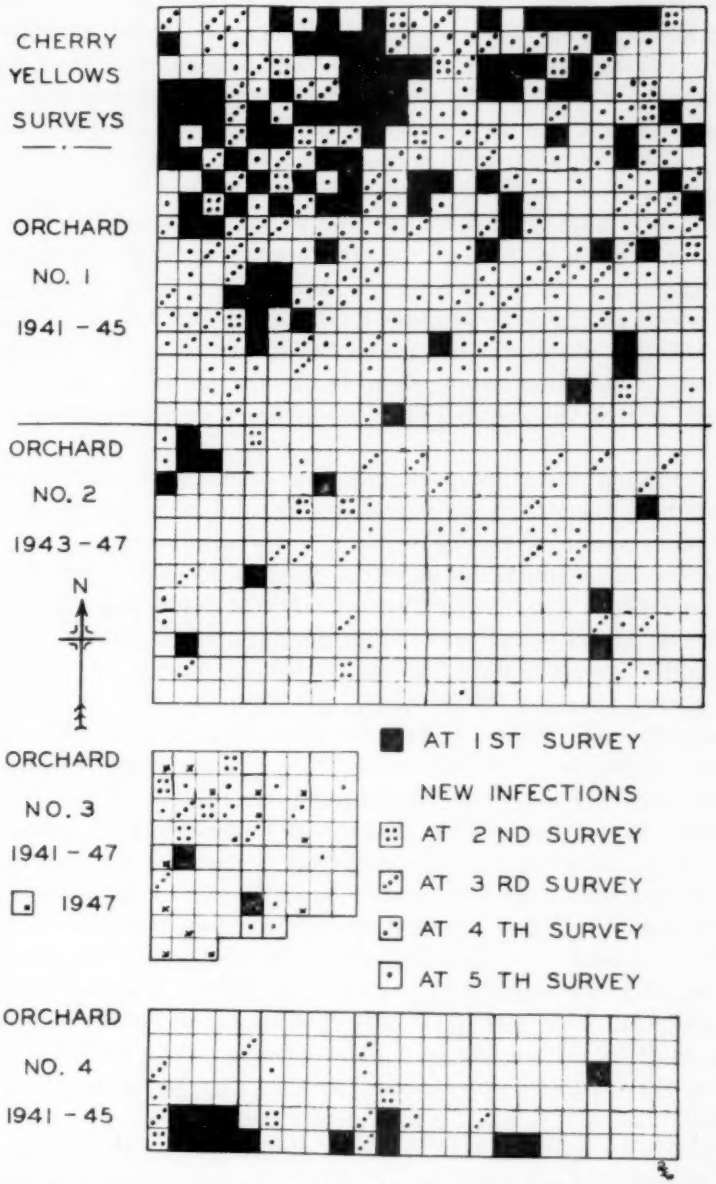

FIG. 1. Diagrammatic maps of four Montmorency cherry orchards showing the distribution of cherry yellows at the time of the first survey and the location of new infections observed during each subsequent survey.

to the west, in which there were scattered a few sour cherries. A group of diseased trees was thus established in the northwest corner, so that con-

ditions here became similar to those in orchard 1. The eastward and southward drift of infection became apparent in the third and subsequent surveys. Orchard 4 is of particular interest, first, because it had the lowest rate of spread, in spite of its being the oldest planting and having the second highest initial incidence, and secondly, because the situation here was almost the reverse of that in orchard 1. The original sources of infection in this orchard were evidently distributed along its southern edge and the spread of infection into the interior of the orchard was comparatively slow (Fig. 1). If it is taken into consideration that, in this region, the prevailing winds are from the west to southwest and that the strongest winds most frequently blow from the northwest, it would appear that the location of infection sources with respect to the prevailing winds, as well as the number and degree of concentration of sources, had some influence on the rate at which cherry yellows spread through the several orchards under survey. That is to say, the disease tended to increase more rapidly where the sources of infection happened to be located on what was usually the windward side of the orchard than where they were located on the leeward side.

The foregoing observations suggest that an insect vector whose movements can be influenced by air currents is likely to be of major significance in the dissemination of cherry yellows in an established orchard. If so, local and seasonal fluctuations in vector populations could exert further influence on rates of spread of yellows in different years and localities.

That the intensity and prevalence of symptoms of cherry yellows depends mainly upon the temperatures prevailing during leaf development in sour cherry trees was demonstrated by Keitt and Moore (8) in greenhouse experiments. Mills (9) has also shown that variation from year to year in the degree of symptom expression of yellows in the orchard can be correlated with the temperatures obtaining during the 30-day period following petal-fall. Consequently, seasonal differences in the annual increase of yellows can be to some extent attributed to the fact that in some years symptom expression may be partially suppressed. In 1944, for example, some new cases could easily have been overlooked and so might not appear in the records until the following year. On the other hand, since symptoms were both prominent and prevalent in 1941 and again in 1945 and only slightly less so in 1942 and 1943, it is reasonable to conclude that the assessment of the yellows status of the various orchards is substantially accurate for the complete survey period, and for most of the individual years concerned.

Necrotic ring spot incidence appears to differ from that of yellows in several respects. In the first place, both initial and current percentages of trees affected with necrotic ring spot were higher in most individual orchards as well as in average and, besides, varied less widely from orchard to orchard (Table 2). This would suggest either that more trees were affected with necrotic ring spot than with yellows at planting time or that

the former disease spread with greater rapidity. At the present stage of investigation there is no way of ascertaining the full effects of the first assumption, except by deduction, though it has some foundation as shown

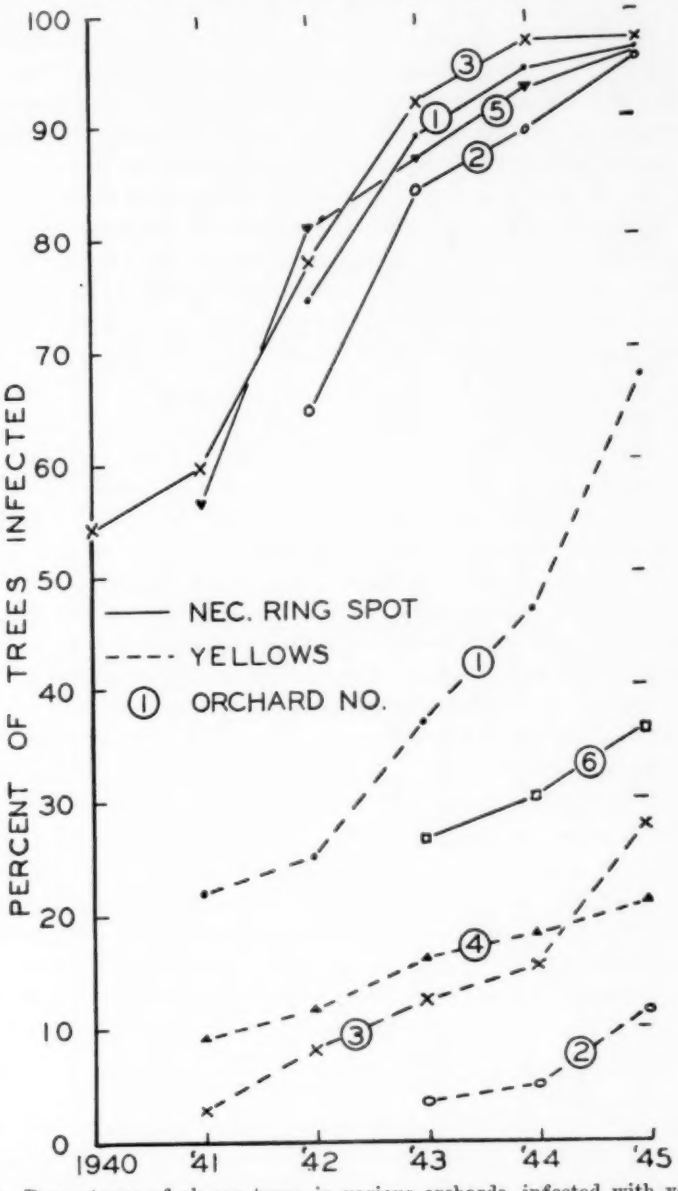

FIG. 2. Percentages of cherry trees, in various orchards, infected with yellows and with necrotic ring spot in successive years. The curves for necrotic ring spot are based on figures for probable spread and adjusted to allow for the occurrence of masking.

by comparison of the number of groups of diseased trees (Table 6). The possibility that necrotic ring spot spreads more rapidly than yellows ap-

TABLE 5.—*Adjusted incidence of necrotic ring spot and the corresponding percentages of previously "healthy" trees becoming diseased*

Orchard No.	Initial incidence	Adjustment	Adjusted incidence						Probable increase (Percentage of "Healthy")					
			1940	1941	1942	1943	1944	1945	1941	1942	1943	1944	1945	Average
1	34.5	+40	—	—	74.5	89.1	94.9	96.7	—	—	57.2	53.2	36.4	48.9 ± 6.4
2	24.6	+40	—	—	64.6	84.1	89.3	95.9	—	—	54.9	32.6	61.3	49.6 ± 8.7
3	29.2 (a)	+25	54.2	59.7	77.9	91.8	97.2	97.2	12.1	44.8	62.4	66.6	0.0	37.2 ± 13.4
	(b)	+27.8	57.0	62.5	80.7	94.6	100.0	100.0	12.9	48.2	71.5	100.0	—	58.2 ± 18.4
5	16.7	+40	—	56.7	80.9	87.0	93.1	96.1	—	55.2	30.8	44.4	40.0	42.6 ± 5.1
6	26.6	+ 0	—	—	26.6	26.6	29.9	36.0	—	—	0.0	4.5	8.7	4.4 ± 2.5

Coefficient of correlation between adjusted incidence and subsequent spread, +0.528 (3–4 per cent), using 3 (a), or +0.683 (< 1 per cent) if orchard 3 were completely infected in 1944 (see 3 (b)). There were no new infections in orchard 3 in 1947.

pears to be confirmed by the data given in table 4, since, on the average, only 8.9 per cent of the "healthy" trees became infected with yellows each year and between 13.2 and 19.0 per cent with necrotic ring spot. These comparisons, however, may not be altogether valid for reasons which are developed later. In the second place, the rates of spread of necrotic ring spot were correlated negatively, instead of positively, with the prevalence of infective sources (Table 3). This inversion of correlation, which is contrary to what would normally be expected of an infectious disease with a relatively high rate of spread, and the contingent progressive falling off of the annual increase in most of the orchards (Tables 2 and 4) are only understandable in view of the masking of necrotic ring spot and of the consequent probability that a considerable proportion of the "healthy" trees were infected before the survey began. Furthermore, if the probable incidence per annum is plotted for individual orchards (Fig. 2), the resultant graphs, except that for orchard 6, take the shape of the upper part of a characteristic sigmoid curve. This type of curve also indicates the probability that necrotic ring spot infection in four of the orchards was approaching the saturation point by 1945.[2] In accordance with this hypothesis, the positions of the curves (Fig. 2) have been adjusted, where adjustment appears to be warranted, to bring the 1945 incidence above 95 per cent (Table 5). The corresponding curves for yellows incidence, and for necrotic ring spot incidence in orchard 6, (Fig. 2) are typical of the lower or middle parts of sigmoid curves, in agreement with the comparatively low or medium degrees of infection observed. The probable spread associated with the adjusted incidence of necrotic ring spot (Table 5) is several times that associated with the observed minimum incidence (Table 4) and shows a significantly positive correlation with the adjusted incidence of the previous year (Table 5). Thus it would appear that the rates of spread, whether as percentages of the total number of trees or as percentages of previously "healthy" trees, is not the same for different levels of infection. It is therefore doubtful if any mean rate of annual increase can be established for either disease. Certainly comparisons should not be made without considering incidence levels. The possible effects of prevailing winds on the spread of necrotic ring spot were obscured by the wide distribution throughout each orchard of groups of trees known to be diseased at the time of the first survey.

DISTRIBUTION OF DISEASED TREES IN ORCHARDS

In their studies of the distribution of psorosis in citrus orchards, Bitancourt and Fawcett (2) devised means of analysing maps compiled in single surveys. By this means they deduced, as they were later able to demonstrate, that psorosis may be transmitted in the orchard by natural root

[2] In support of the hypothesis that 4 of the 5 orchards were close to the saturation point for necrotic ring spot, it may be noted that in 1947 the incidence of new infections ranged from 0.0 to 0.5 per cent (of totals) in orchards 1, 2, 3, and 5, but was 10.8 per cent in orchard 6.

grafts. In their method of analysis, the average number of diseased trees at certain distances around each diseased tree was compared with the average number of diseased trees at the same distances around healthy trees. This was done in order to disclose possible patterns of spread from sources introduced unknowingly into the orchard at planting. The distances involved were 1 L, 1.4 L, 2 L, and 2.8 L, where L is the planting distance. In the present studies 2.2 L and 3 L + were also included. The relative positions of trees at these distances from a central tree are shown in figure 3. However, the method of analysis was modified to take advantage of data obtained in a series of surveys. From information of this type it was possible to calculate for each disease not only the percentage of diseased trees at each distance from the nearest source, but also the percentage of "healthy" trees both available for later infection and becoming infected at these respective distances. It should be understood

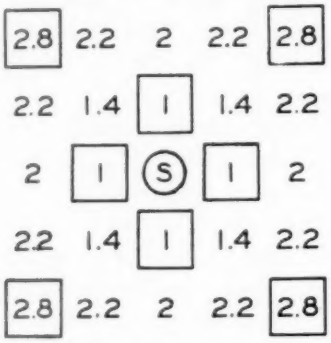

FIG. 3. Location of trees at various distances 1 L to 2.8 L from a central tree, S, where L = planting distance. All trees outside this pattern are classified as 3 L +.

that, in presenting the results of this analysis it is intended to show the probability of occurrence of spread over various minimum distances and not to imply that spread invariably followed the patterns indicated.

The distributions of diseased and "healthy" trees, with respect to the nearest diseased tree, in the various orchards at the time of the first survey are presented in table 6. The relatively high percentages of diseased trees adjacent to each other are interpreted as evidence of short distance spread taking place before the surveys began and resulting in groupings of affected trees, particularly where incidence was low, for example, the groupings of initial yellows infections in orchard 4. It should also be observed that the opportunities for short distance spread to "healthy" trees become more and more prevalent as more trees become infected (Table 6). High percentages of diseased trees at 3 L + may arise either from spread or from the distribution of trees infected when set out, for example, yellows in orchards 2 and 3.

The probable distribution of spread can be determined with greater accuracy by considering the relative frequency with which new cases occur at various distances from the nearest source of infection in the second

TABLE 6.—*Percentages of diseased and of healthy trees to be found at different distances from the nearest diseased tree, and the number of groups of diseased trees at the time of the first survey*

Orchard No.	Initial incidence	No. of groups	Distribution of diseased trees at						Distribution of "healthy" trees at					
			1 L[a]	1.4 L	2 L	2.2 L	2.8 L	3 L+	1 L	1.4 L	2 L	2.2 L	2.8 L	3 L+
Cherry Yellows														
1	22.0	20	75.8	14.7	6.3	1.1	0.0	2.1	43.9	17.8	12.7	11.6	3.3	10.7
2	3.5	6	30.0	10.0	20.0	0.0	0.0	40.0	11.2	9.7	9.3	16.2	5.4	48.2
3	2.8	2	0.0	0.0	0.0	0.0	0.0	100.0	11.4	11.4	8.6	10.0	4.3	54.3
4	9.4	4	84.6	0.0	7.7	0.0	0.0	7.7	17.6	9.6	12.8	14.4	6.4	39.2
5	45.5	6	80.0	20.0	0.0	0.0	0.0	0.0	72.3	13.9	11.1	2.7	0.0	0.0
6	27.7	13	88.0	8.0	2.0	2.0	0.0	0.0	58.4	11.5	8.5	8.5	3.1	10.0
7	18.8	20	68.5	11.0	8.2	5.5	4.1	2.7	47.2	19.7	12.1	8.9	3.5	8.6
8	26.8	21	70.0	13.3	13.3	3.3	0.0	0.0	59.1	19.5	12.2	5.5	0.6	3.1
9	100.0	?	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Necrotic Ring Spot														
1	34.5	38	83.9	10.7	4.7	0.0	0.7	0.0	76.7	14.8	5.3	2.5	0.0	0.7
2	24.6	19	77.5	12.7	7.0	1.4	0.0	1.4	61.3	23.0	8.8	4.6	0.5	1.8
3	29.2	9	57.2	23.8	0.0	9.5	0.0	9.5	70.6	21.6	3.9	3.9	0.0	0.0
5	16.7	4	63.6	27.3	0.0	9.1	0.0	0.0	41.8	21.8	9.1	7.3	1.8	18.2
6	26.6	16	75.0	16.7	0.0	6.2	2.1	0.0	61.4	22.7	10.6	4.5	0.0	0.8

[a] L = planting distance between trees. (See figure 3.)

and subsequent surveys (Fig. 4). New yellows infections can appear at any of the specified distances, but the frequency with which they do is not proportional to the available opportunities but rather drops off markedly as the distance from possible sources increases (Fig. 4). Records for individual orchards, with a few variations in details, also tend to conform to the general rule. It may be concluded therefore that yellows tends to spread more readily to adjacent trees than to the more remote ones. However, in spite of this tendency, 47.8, 39.5, and 51.0 per cent of the healthy trees at present adjacent to diseased trees in orchards 1, 3, and 4,

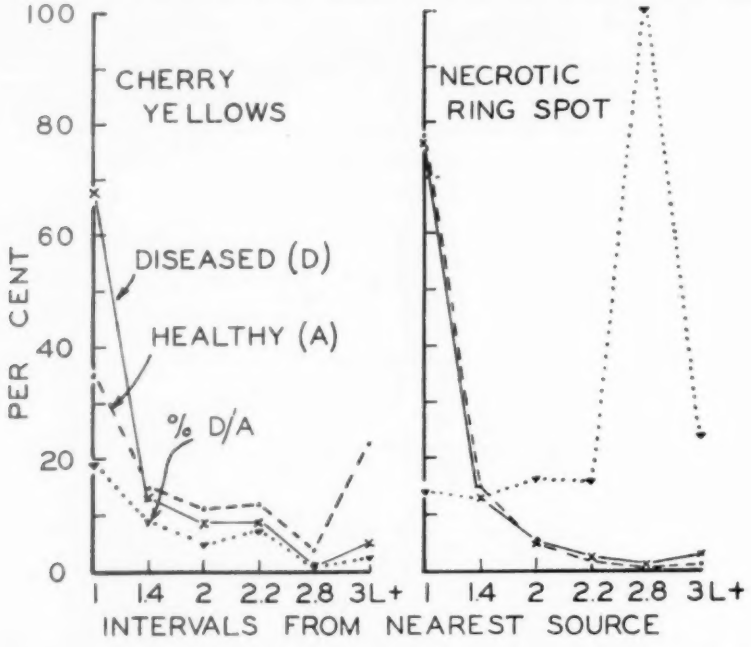

FIG. 4. Percentages of "healthy" trees (A) available for infection, percentages of new cases (D) and the percentage of healthy trees (D/A) becoming diseased, at each interval from the nearest source.

respectively, have occupied that position for three or more years. It would thus appear that transmission of yellows by natural root grafts (2), though a possibility, is not a common occurrence. The evidence obtained from the analysis of distribution of spread, taken as a whole, supports the view that cherry yellows is disseminated in the orchard mainly by a vector, the movements of which, besides being influenced by wind, are habitually restricted to single trees, but which may migrate occasionally from one tree to the next and less frequently to more distant ones. It seems reasonable also to conclude that the vectors are neither numerous nor prolific, otherwise a greater frequency of tree to tree migration and consequently a more rapid increase of the disease could be expected. It might be pointed out that some species of leaf hopper and aphids could fulfill the

necessary requirements. A somewhat similar conclusion was reached by Keitt and Clayton (7) who, after an entirely different approach to the problem, have reported the probability that leaf hoppers (Cicadellidae) may be vectors. Investigations are now in progress to determine and establish these points.

If the analysis of spread of necrotic ring spot is taken at its face value, the results indicate that this disease has a wider range of spread than yellows. The curve showing the percentage of new cases at each distance from the nearest diseased tree is virtually identical with the corresponding curve for "healthy" trees available for infection (Fig. 4). This in itself suggests that the frequency of new infection at any distance from source is directly proportional to the number of opportunities available. The same conclusion may be drawn from the curve for the percentage of "healthy" trees becoming diseased in each category. It may be noted in passing that the 100 per cent point reached at 2.8 L in this curve is purely fortuitous since there were only two trees in the category and both of them happened to become infected. Before accepting the above-mentioned conclusion and its corollary that different types of vector are responsible for the spread of necrotic ring spot and yellows, it should be remembered that the same distribution in the orchard could be obtained with a more limited range of speed, providing a number of apparently healthy but actually diseased trees were scattered through each of the orchards. Since there is good evidence, already submitted, for believing this condition to be fulfilled, it is considered to be the major part of the explanation for the apparent distribution of spread of necrotic ring spot. Thus there is at present as much reason for concluding that the means of dissemination of necrotic ring spot are very similar to, if not the same as, those of yellows, as there is for concluding that they are different.

OVERLAPPING INCIDENCE

The fact that yellows has seldom if ever been transmitted unaccompanied by indications of the presence of necrotic ring spot has given rise to the idea either that the two sets of symptoms may be phases of the same disease or that symptom expression of yellows in sour cherries may occur only when the tree is infected by a mixture of the yellows and necrotic-ring-spot viruses. Accordingly the frequencies with which each disease appears alone and in combination and of the orders in which infection with each disease occurred in mixture have been determined (Table 7). In most of the orchards more than half of the total number of trees known to be diseased were infected with necrotic ring spot alone and considerably less than half appeared to be infected with yellows alone. Also, about two-thirds of the trees known to have necrotic ring spot did not have yellows while half the trees that had yellows were not known to have necrotic ring spot. Since a substantial proportion of the trees showing symptoms of only one disease had been infected for four or more years, it would

TABLE 7.—*Frequency of association of yellows and necrotic ring spot and of order of appearance of infections*

Combination	Orchard 1		Orchard 2		Orchard 3		Orchard 5		Orchard 6		Total	
	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
N.R.S. only	92	*23.9*	161	*83.1*	39	*66.1*	....	.....	....	.....	292	*45.8*
N.R.S. first	135	*35.2*	15	*7.7*	9	*15.2*	....	.....	....	.....	159	*25.0*
In same year	13	*3.4*	.....	.....	...	.....	....	.....	....	.....	13	*2.0*
Y first	6	*1.6*	3	*1.5*	3	*5.1*	....	.....	....	.....	12	*1.9*
Order not known	13	*3.4*	1	*0.5*	1	*1.7*	....	.....	....	.....	15	*2.4*
Y only	125	*32.5*	14	*7.2*	7	*11.9*	....	.....	....	.....	146	*22.9*
Total diseased trees	384	*100.0*	194	*100.0*	59	*100.0*	....	.....	....	.....	637	*100.0*
N.R.S. only	92	*35.5*	161	*89.4*	39	*75.0*	24	*63.2*	60	*84.5*	376	*62.7*
N.R.S.—Y	167	*64.5*	19	*10.6*	13	*25.0*	14	*36.8*	11	*15.5*	224	*37.3*
Total N.R.S.	259	*100.0*	180	*100.0*	52	*100.0*	38	*100.0*	71	*100.0*	600	*100.0*
Y only	125	*42.5*	14	*42.4*	7	*35.0*	16	*53.3*	39	*78.0*	201	*47.3*
Y—N.R.S.	167	*57.5*	19	*57.6*	13	*65.0*	14	*46.7*	11	*22.0*	224	*52.7*
Total Y	292	*100.0*	33	*100.0*	20	*100.0*	30	*100.0*	50	*100.0*	425	*100.0*

appear that necrotic ring spot is not necessarily a phase of yellows. That yellows can occur without necrotic ring spot is not deducible from the percentage of trees showing only yellows symptoms because necrotic ring spot may be masked in many or all such cases. But it is indicated as a possibility by the small percentage of cases in which yellows symptoms were observed for two or three years before the appearance of severe or acute symptoms of necrotic ring spot. The evidence on the whole favors the theory that the two diseases are caused by different viruses and can occur independently but the possible effect of necrotic ring spot on symptom expression of yellows has yet to be completely determined, if and when the casual viruses are separated.

Because of the potentially wide distribution of necrotic ring spot at planting time, it is to be expected that new yellows infections in orchards would, in the ordinary course of events, occur more frequently in trees already affected with necrotic ring spot than in healthy ones. In 26.4 per cent of the cases where necrotic ring spot preceded yellows, acute symptoms of the two diseases appeared in successive years. Some of these cases should perhaps be regarded as simultaneous or coincidental infections with the two viruses, since yellows symptoms are known to be sometimes delayed until the second year after infection. For the same reason, in some of the cases where acute symptoms of both diseases appeared in the same year, yellows infection could have occurred first. Infections with both viruses in the same year could be brought about either simultaneously by the same vector or accidentally by different vectors, but further evidence is necessary to substantiate either conclusion.

SUMMARY

Annual surveys have been conducted in several sour cherry orchards over a period of years to determine the incidence, the distribution of old and new cases, and the rates and conditions of spread of cherry yellows and necrotic ring spot. The problem is complicated by the tendency for the latter disease to become masked in course of time. Analysis of the data indicates that a greater percentage of trees are likely to be infected with necrotic ring spot than with yellows when an orchard is set out, that rates of spread are largely determined by initial incidence and by the relative position of affected and "healthy" trees at planting, and that little fundamental difference in the rates and manner of spread of the two diseases can be expected even when apparent differences are considerable. Cherry yellows tends to spread more frequently to adjacent than to more remote healthy trees and its dissemination appears to be influenced to some extent by prevailing winds. It is suggested that yellows may be carried from tree to tree mainly by an insect vector which only infrequently moves from one tree to another and whose movements at such times may be affected by air currents.

In each orchard surveyed, some trees showed symptoms of only one of

the diseases, some, symptoms of both, and some were apparently healthy. Since the symptoms of yellows appear annually and those of necrotic ring spot may become masked, trees showing only the latter may be presumed yellows-free, but trees showing only yellows symptoms may also have necrotic ring spot. In cases where trees are known to have both diseases, necrotic ring spot most frequently preceded yellows, but sometimes yellows appeared before or at the same time as necrotic ring spot.

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BROWN STEM ROT OF SOYBEAN

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Brown stem rot of the soybean (*Glycine max* (L.) Merrill) was first discovered in 1944 in central Illinois (1). Since that time it has become one of the most important diseases of the soybean crop throughout Illinois and is becoming increasingly important in Indiana, Iowa, Missouri, and Ohio. A short description of the disease has been given by Allington (1). Presley and Allington (3) identified the causal organism as a member of the genus *Cephalosporium*, but made no species identification. It is the purpose of this paper to describe the causal organism and to report the results of current studies on the disease.

SYMPTOMS

The first symptom of brown stem rot usually appears in July or early August in Illinois. At this time infected plants show no external evidence of the disease. When the stems are split longitudinally, however, a brown coloration of the pith and vascular elements is plainly visible, extending upward from the base of the stem (Fig. 1, E). In seasons which are unfavorable for disease development, only the internal stem symptoms may be found. At first the browning extends but two or three inches above the soil line, more prominent at the nodes. Later, under conditions favorable for the disease, the browning becomes continuous throughout the stem. External symptoms of the disease usually appear in late August or the first week in September when a sudden blighting and drying of the leaves is noted. The onset of this phase is so rapid that the farmer often suspects frost damage. The leaf tissues adjacent to the veins remain green while the interveinal portions die (Fig. 1, A). From a distance a badly infected field appears brown as contrasted with the yellow-green of a normally maturing field.

The severity of the final effects of brown stem rot depends somewhat upon weather conditions at the time of harvest. If warm, dry weather prevails, infected soybeans may be harvested with little loss. However, under conditions of low temperatures before harvest time the weakened stems lodge badly and substantial losses occur because of mechanical difficulties in harvesting. When harvest is delayed by unfavorable weather or other factors, losses are greatly accentuated since the weakening of the stems with subsequent lodging continues until harvest.

THE PATHOGEN

Macroscopic Characters. The macroscopic characters of the pathogen are, unless otherwise stated, those observed in 14-day-old cultures on 2 per

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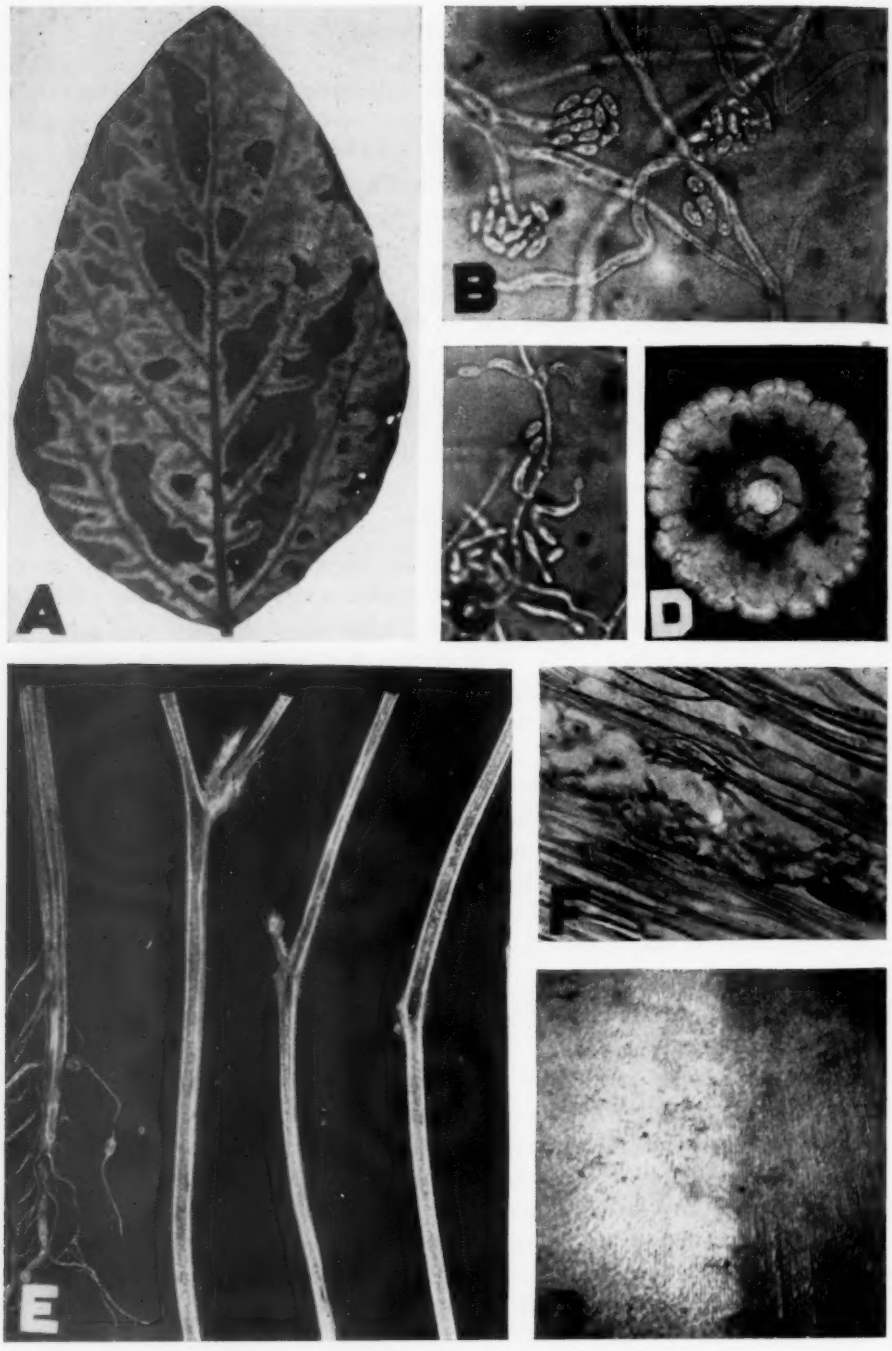

FIG. 1. Symptoms of brown stem rot of soybeans and characters of the causal organism (*Cephalosporium gregatum* n. sp.) observed in pure culture. A. Leaf symptoms observed in late fall. B. Conidial heads in pure culture produced on soybean-stem agar. C. Aggregated conidiophores and conidia observed in pure culture on soybean-stem agar. D. Three-weeks-old culture on potato-dextrose agar. E. Brown coloration in vascular system and pith of naturally infected field plant. F. Longitudinal section of vessel in stem showing fungus mycelium in vessel. G. Aerial view (altitude 600 ft.) taken in mid-September showing sharp line of demarcation between diseased and healthy portions of a soybean field, resulting from crop rotation. Right, diseased; left, healthy.

cent potato-dextrose agar at 20° C. Mycelial growth is slow, producing a flat, dense, approximately orbicular mat, white to putty-colored or gray, with radial folds that become more prominent with age (Fig. 1, D). The margin is variable, but usually finely lobed. In 7- to 14-day-old cultures the mycelial mat is slightly floccose. Through the bottom of the Petri dish the cultures appear smooth, tan with a gray margin, and sometimes slightly concave. The diameter of 14-day-old cultures, growing from 4-mm. mycelial disks at 20° C., varies from 2.0 to 2.4 cm.

On soybean-stem agar[2] the organism produces a thin, spreading, transparent mycelial growth, inconspicuous by reflected light. In cultures 3–4 weeks old concentric rings can be seen by transmitted light. On this medium the diameter of 14-day-old cultures varies from 2.3 to 2.9 cm.

Microscopic Characters. The hyphae are hyaline, septate, branched, and 1.2–4.7 μ in diameter. In cultures more than ten days old the hyphae frequently form aerial or decumbent fascicles. On soybean-stem agar the conidiophores are usually short, 4–15 μ long, but occasionally reach 25 μ in length. They are hyaline, continuous or septate, simple or branched, straight or club-shaped. They may be produced at any point on the fertile hyphae, usually appearing in groups (Fig. 1, C). When borne at the tip of a fertile hypha, the conidiophore is not well differentiated. Conidia are produced in succession at the apex of the conidiophore to form an irregular head, and are non-catenulate (Fig. 1, B). When the heads are produced in close proximity they aggregate to form large, irregular spore masses, either on the surface of the agar or on aerial hyphae. These spore masses disintegrate instantaneously upon contact with water unless they have undergone considerable drying as would be the case in old cultures. The conidia are non-septate, ovoid to elliptical, hyaline, 1.7–3.4 × 3.4–7.6 μ. Spore heads or conidiophores have not been observed in or on soybean plants, but the conidia have been found in the exudate taken from infected cut stems to which water pressure had been applied. The conidia produced within the soybean stems are typically larger (3.4–4.3 × 6.8–9.4 μ) than those produced in pure culture. Conidia germinate by a single germ tube from one end or occasionally by two germ tubes, one from each end of the spore.

Cultural Studies. The organism has shown no noticeable genetic variation in pure culture over a period of three years. Sectoring or changes in pathogenicity have not been detected, and all the isolates taken from naturally infected plants for a period of three years appear to be identical. When cultures were started from finely macerated mycelium, variations in color from white to dark brown occurred but subsequent transfers yielded only the normal type.

Sporulation does not occur on potato-dextrose agar in sufficient quantities to be detected except by a critical examination of washings made from such cultures. By this method a few spores sometimes can be found, as reported

[2] Soybean-stem agar was prepared by adding an extract of 25 gm. macerated green soybean stems in 100 ml. water to 1 liter of 2 per cent agar.

by Presley and Allington (3). A large number of vegetable decoctions in 2 per cent agar, with and without dextrose added, were tested as media for satisfactory sporulation. Conidia were produced on string-bean agar, rice-polish agar, cucumber agar, and soybean-stem agar. The addition of 2 per cent dextrose to the above media inhibited sporulation. Since spore production was best on the soybean-stem agar, it was used almost exclusively for the microscopic studies of the pathogen involving spore production. Spores appeared on soybean-stem agar cultures after five days at 20° C.

The effect of temperature upon growth rate was studied on potato-dextrose agar and soybean-stem agar. The minimum temperature for growth is below 8° C., the optimum 22°–24° C., and the maximum above 30° C. The optimum temperature for sporulation in culture is between 15° and 20° C., definitely below the optimum for mycelial growth. Sporulation has never been observed at temperatures above 28° C.

Germination of conidia was observed after 24 hours in distilled water and in concentrated soybean-stem juice at 15°, 18°, 20°, 21°, 24°, 25°, 27°, and 30° C. The highest percentage of germination was 88 in soybean-stem juice. The optimum temperature range was 21°–25° C. At 15° C. germination was 0–2 per cent; at 30° C. it was 0–9 per cent.

Host Range Studies. Extensive tests have not been made in studying the host range of the brown stem rot organism. A few species of cultivated plants were inoculated, however, by making stem punctures near the base of the stem and inserting mycelium. This method has never failed to result in heavy infection of soybean. The following species were tested for susceptibility: tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), red kidney bean (*Phaseolus vulgaris* L.), castor bean (*Ricinus communis* L.), mung bean (*Phaseolus aureus* Roxb.), pea (*Pisum sativum* L.), sunflower (*Helianthus annuus* L.), red clover (*Trifolium pratense* L.), and corn (*Zea mays* L.). The disease developed only in mung bean. Not only were symptoms absent in the remaining species, but in repeated trials the organism was never reisolated from the inoculated plants. In all cases soybeans were used as a check on the technique used and infection and recovery of the pathogen always occurred.

Comparison of the Cultural and Parasitic Characters of Cephalosporium acremonium Corda and the Brown-Stem-Rot Organism. Reddy and Holbert (4) have reported the pathogenicity of *Cephalosporium acremonium* Corda to corn in the Midwest, consequently the brown-stem-rot fungus was compared very closely with it.

In culture the two organisms are distinct. *Cephalosporium acremonium* on potato-dextrose agar produces a white, floccose, orbicular mycelial mat that later develops a typical pinkish tinge. As stated earlier, the brown-stem-rot fungus produces a flat, dense, radially folded mat of putty-colored mycelium. On potato-dextrose agar *C. acremonium* sporulates profusely in 5–7 days, while the soybean pathogen produces few or no spores. On soy-

bean-stem agar both organisms produce a thin, transparent mycelial growth and sporulate abundantly. The rate of growth of *C. acremonium* is about twice that of the soybean parasite on both media. Detailed differences in morphology of the two species are described under the section on taxonomy.

Thirty different corn hybrids were inoculated in the seedling stage with the two organisms by the stem puncture method. Although no definite disease symptoms were produced by either organism, the corn was definitely susceptible to *Cephalosporium acremonium*. This organism grew within the plants and was reisolated after six weeks from 75 per cent of the plants inoculated. On the other hand, the soybean parasite was not reisolated from the corn in repeated attempts. Seventeen soybean plants (var. Bansei) were inoculated with *C. acremonium* and a similar number with the soybean parasite. *C. acremonium* produced no symptoms and was only recovered in 2 per cent of the platings of tissue at the points of inoculation. The soybean parasite caused the majority of the plants to develop typical brown stem rot symptoms and 90 per cent of the platings were positive.

Taxonomy.[3] The brown stem rot pathogen apparently belongs in the genus *Cephalosporium* Corda of the Moniliaceae. No other organism of this genus has previously been described as a parasite on soybean. The description of this organism does not seem to conform to the descriptions of the *Cephalosporium* species listed by Buchanan (2) and Saccardo (5, 6, 7, 8, 9, 10, 11). *C. acremonium* Corda, a parasite on corn in Illinois (4), resembles the soybean pathogen somewhat on the basis of spore shape. However, the conidia of *Cephalosporium acremonium* (1–1.8 × 2.5–6 μ)[4] are appreciably narrower and shorter than those of the soybean parasite. *C. acremonium* produces well-separated conidial heads on erect conidiophores. On favorable media the soybean pathogen produces short, straight or club-shaped conidiophores, singly or more frequently in groups. This latter behavior results in the most outstanding characteristic of the fungus, suggesting the specific name. The heads, produced in close proximity to each other, aggregate to form large, irregular masses of spores at the surface of the agar or slightly above it. This differs markedly from anything described for any known member of the genus *Cephalosporium*. The brown stem rot pathogen, however, appears to be more closely related to that genus than to any other. Accordingly, the name *Cephalosporium gregatum* n. sp. is proposed.

Cephalosporium gregatum n. sp.

Mycelial mat dense, flat, orbicular, white, later becoming putty-colored or gray, with conspicuous radial folds; sterile hyphae hyaline, septate, branched, 1.2–4.7 μ broad, often forming aerial fascicles or tufts; fertile hyphae usually 1.2–2.0 μ broad, aerial or decumbent; conidiophores short, usually 4.2–15 μ but occasionally up to 25 μ in length, simple or occasionally branched,

[3] Grateful acknowledgment is made to Dr. M. P. Backus, University of Wisconsin, for mycological assistance.

[4] Measurements of Fresenius, Saccardo, Oudemans and Koning, Bainier, and Ciferri (4).

straight or club-shaped, usually continuous but occasionally septate, sometimes produced singly but usually in clusters, either terminally or along the fertile hyphae; conidia ovoid to elliptical, 1.7–3.4 × 3.4–7.6 μ in culture, up to 4.3 × 9.4 μ in the host, hyaline, continuous, capitate, not catenulate; conidial heads irregular, aerial or decumbent, usually aggregating to form mound-like masses of spores close to or at the surface of the medium.

Habitat: In roots and stems of living soybean plants (*Glycine max*) and in soils in Illinois, Indiana, Iowa, Kentucky, Missouri, and Ohio, U.S.A.

Mycelium in culturis tegeticulam compactam decumbentemque formans, orbicularem, primo albam denique griseam colore, in rugas radiantes conspicuas plicatam; hyphis sterilibus hyalinis, septatis, ramosis, 1.7–4.7 μ crassis, saepe in fasciis aeriis protrudentibus; hyphis fertilibus aeriis vel decumbentibus, plerumque 1.2–2.3 μ crassis; conidiophoris brevibus, plerumque 4.2–15 μ, aliquando ad 25 μ longe, simplicibus vel ramosis, rectis vel clavatis, continuis tamen nonnumquam septatis, aliquando extremitatibus saepius ullis intermediis locis hypharum fertilium in caespitibus locatis; conidiis ovoideis vel ellipticis, 1.7–3.4 × 3.4–7.6 μ in culturis, ad 4.3 × 9.4 μ in hospitibus, hyalinis, continuis, capitatis, non catenulatis; capitibus irregularibus, aeriis vel decumbentibus, plerumque grumulos conidiorum summis mediis formatibus.

Habitat in radicibus et caulibus plantarum vivarum (Glycine max) et in terris in Illinois, Indiana, Iowa, Kentucky, Missouri, et Ohio, U.S.A.

HOST-PARASITE RELATIONS

It has been repeatedly demonstrated that the pathogen can enter the host through artificial wounds at the base of the stem. In the greenhouse, plants growing in naturally infested soil have become infected without artificial wounding. In steamed sand, infection was obtained by pouring inoculum around the roots or by dipping the roots into the inoculum before transplanting. It appears that in fields in Illinois penetration occurs early in the summer, perhaps 3–4 weeks after planting, and that the main and lateral roots are the most common points of entry. Microscopic examinations at weekly intervals throughout the growing season have consistently revealed the presence of the organism in the vessels of the main root prior to stem invasion. The amount of mycelium in the vessels varies from a few strands to a solid mass of hyphae sometimes occupying almost the entire lumina of the vessels (Fig. 1, F). The fungus appears to migrate through the walls between the vessels. In actively growing plants it is predominantly a vascular parasite and is found only occasionally in the pith. The organism progresses upward through the vessels of the stem, lateral branches, and petioles, and can be found near the tips of the plants at maturity. In mature plants mycelium is found in the pith more abundantly than at any previous stage of plant development.

It does not seem likely that the organism overwinters in stems. When diseased plants were dried and kept in the greenhouse for 3 months, it was

not possible to recover the organism. Numerous attempts to isolate the fungus from overwintered stems in the field have likewise failed. It has been demonstrated, however, that the fungus can exist for more than a year in moist soil stored in the greenhouse. The slow growth habit of this organism has not permitted isolation, as yet, directly from the soil. Plants grown in infested soil under the proper environmental conditions readily become infected and thereby supply a reliable criterion for the existence of the parasite in the soil. Seed from diseased plants has been plated out repeatedly but no indication has been found that the organism is seed borne.

Effect of Temperature on Disease Development. The effect of soil temperature on infection and development of the disease was studied in the greenhouse. Naturally infested soil was placed in earthenware containers and maintained at the following temperatures: 10°, 15°, 20°, and 25° C. Six pots with at least four seeds planted in each pot were used at each temperature. The experiment has been repeated twice, using four pots instead of six as in the first test. The air temperature of the greenhouse varied from 15° to 26° C. during the tests. Only a trace of infection was detected at each of the temperatures. The plants were grown to within two weeks of maturity in most cases. Although these results are inconclusive, it is believed that the disease is not particularly responsive to different soil temperatures.

The effect of air temperature on the incidence and development of the disease was also studied in the greenhouse. Three controlled air-temperature chambers at 15°, 21°, and 27° C. were used. In several tests the Lincoln and Bansei varieties of soybeans, six to ten inches tall, were artificially inoculated near the ground level by stem punctures. Small amounts of mycelium from a non-fruiting culture on potato-dextrose agar were inserted into the punctures. After two to three weeks at 15° C. air temperature, the internal browning of the stems was evident 8–10 inches above the point of inoculation, and typical leaf symptoms of the disease were observed. At 21° C. progress of infection was almost inhibited and at 27° C. no spread of the fungus was observed. Platings from these stems two to three inches above the point of inoculation failed to yield the organism. Isolations within one inch of the point of inoculation in the plants held at 21° and 27° C. usually were positive. Lowering of the temperature to 15° C. after incubation at 21°–27° C. always resulted in a severe attack of the disease within two weeks.

In two tests inoculum from soybean-stem agar bearing abundant conidia was compared with inoculum from potato-dextrose agar bearing no conidia by means of basal-stem-puncture inoculations. In plants held at air temperatures below 21° C. the inoculum bearing conidia caused typical symptoms and death of the plants before symptoms were evident in the plants inoculated with the potato-dextrose agar cultures. Noninoculated plants in the same environment remained healthy. All the plants held above 21° C.

remained symptomless regardless of the type of inoculum used. However, when the temperature was lowered below 21° C., all the inoculated plants soon developed symptoms of the disease. The time required for symptoms to develop in this case was noticeably different with the two inocula used. It was evident that the presence of spores was a major factor in the time required for development of the disease in plants held at low temperatures from the time of inoculation.

Conidia were produced in the vascular system of naturally or artificially infected plants held at temperatures below 21° C. This was demonstrated by forcing water through the cut stems under considerable pressure and examining the exuded water microscopically. Also by this method suspensions of spores from pure cultures have been passed through sections of healthy stems, indicating the possibility that spore production and movement may be of importance in the spread of infection within the plant.

EFFECT OF CROP ROTATION ON THE INCIDENCE OF BROWN STEM ROT IN ILLINOIS

The external plant symptoms in the later stages of the brown stem rot are so conspicuous that one may readily identify them by aerial observations. The over-all brown color caused by the dead leaves contrasts sharply with the yellow-green of normally maturing fields. Consequently, in September of 1946 an aerial survey of brown stem rot was made. One of the most conspicuous characters of the disease from this vantage point was that certain fields were only partly diseased, and a sharp line of demarcation separated the diseased and healthy portions of the fields (Fig. 1, G). Such fields were located on a detailed map while in the airplane and the lines of demarcation roughly sketched. The farm was later visited by car, the diagnosis of brown stem rot verified, and the farmer interviewed in regard to history of the diseased and healthy portions of his field.

Without exception, the information gained by this method indicated a crop rotation effect upon the incidence of the disease. Each of these fields served as two experimental plots, providing an accurate history could be obtained. Within a very short period of time (2 weeks) 35 such fields were investigated. Completely reliable histories were available on 15 of these fields for a period of at least four years. A four-year rotation of corn, soybeans, oats, and clover controlled the disease completely on the farms studied, while portions of the same fields which in previous years had been farmed separately and therefore had different crop histories were severely infected. Typical examples of rotation histories on severely diseased fields were as follows: (1) corn, soybeans, corn, soybeans; (2) soybeans, corn, corn, soybeans; or (3) clover, corn, soybeans, soybeans. Continuous cropping to soybeans is of course very rare, but a few cases were found and invariably severe stem rot was present. In many cases the healthy portion of the field was growing its first crop of soybeans. The disease was not found where

a previous crop of soybeans had not been grown within the previous three years. On the basis of these observations, it seems reasonable to conclude that one method of controlling the disease in Illinois is to practice a four-year rotation.

DISCUSSION

Brown stem rot of soybean is becoming of great importance in the areas of intensive soybean production in midwestern United States. The organism is probably indigenous in view of the fact that it is non-parasitic to the other common crops of the area, that it is soil-borne and definitely affected by the crop rotations practiced in recent years, that it can grow saprophytically, and that the disease is unreported elsewhere. Soybean culture in the area affected is relatively new, although intensive; in view of the present knowledge, one may believe that its sudden appearance and devastation is primarily the result of cropping practices of the area.

In view of the accumulated knowledge to date, one might logically expect the disease to spread northward from its present location with continued cultivation of the soybean crop in northern areas. It is doubtful, in view of the critical low temperature requirements, that the disease will become of importance in the southern soybean-producing areas of the United States.

The lack of response to soil temperatures and the striking response to air temperatures noted in this study are of special interest. These results are somewhat correlated with the behavior of the organism in pure culture. Mycelial growth was optimum around 22°–24° C., while spore production was optimum below 20° C. The presence of abundant spores in infected plants growing at low temperatures, the decidedly greater potency of inoculum bearing spores, and the demonstration that the spores move readily through the vascular system suggests an explanation for the rapid development of the disease in the upper plant parts under cool conditions. Further work will be necessary to prove this hypothesis, however. It is possible that the spread of the organism, which in pure culture, at least, is a very slow grower, within the upper plant parts is dependent upon the conidia that may move up the stem in the transpiration stream.

Infection from the soil in the roots and basal stem portions apparently proceeds very slowly under high temperature conditions of mid-summer, but with the occurrence of a few cool days in the autumn enough conidia are produced to spread rapidly up the stem. This possibly would also explain the rapid onslaught of the disease in the fall.

SUMMARY

The *Cephalosporium* causing brown stem rot of soybeans is described and the name *Cephalosporium gregatum* n. sp. is proposed.

A limited survey revealed no hosts other than soybean and mung bean for this pathogen.

Air temperatures are of critical importance in the development of the disease, but soil temperatures were not found to be of significance. Air temperatures below 21° C. were essential for the rapid spread of the organism up the stem and the development of typical leaf symptoms. A possible explanation of the mechanism of this reaction is advanced, based upon the production of conidia within the vascular system.

Detailed studies of disease epidemics, aided by aerial observations, revealed a close relationship between the incidence of this disease and crop rotations practiced in the areas most affected. A crop rotation system which provides for three successive annual crops other than soybeans between soybean plantings is recommended for control of brown stem rot in Illinois.

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SUMMARY OF COOPERATIVE EXPERIMENTS ON TREATMENT OF FLAX SEED[1]

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This cooperative investigation, commenced under the auspices of The American Phytopathological Society's Committee for Coordination in Cereal and Vegetable Seed Treatment Research, was begun in 1944. Initially embracing trials at 8 stations in the United States and 2 in Canada, the project has expanded to include trials at 21 stations. The locations of these stations and the names of the cooperators are listed in table 1.

The present report, covering the 3-year period 1944–46, deals with cooperative tests of some standard and some new seed disinfestants or protectants applied to "sound" and to "fractured" flax seed (Fig. 1). The nature of the injury to the seed suggests that the materials tried could function in these tests only as protectants. In view of the diverse conditions under which the tests were made, it is hoped that the findings reported here will be applicable over a wide area of this continent.

MATERIALS AND METHODS

The "sound" seed used in these experiments was derived from reasonably healthy crops of Royal flax in which the plants were threshed by passing them between the rollers of an ordinary clothes wringer. The amount of fracturing in this seed ranged from 0 to 4 per cent. Seed containing 31 to 53 per cent of "fractured" seeds was obtained by threshing parts of the above crops with a steel stationary thresher.

The preparations applied to flax seed in the present tests were as follows: New Improved Ceresan (5 per cent ethyl mercury phosphate), Semesan Jr. (1 per cent ethyl mercury phosphate), Dubay 1452F (7.7 per cent ethyl mercury p-toluene sulfonanilide), Spergon (98 per cent tetrachloro parabenzoquinine + 1 per cent phosphate), and Arasan (50 per cent tetramethyl thiuramdisulphide). They were generally applied at the rates recommended by their manufacturers. Several rates of application of New Improved Ceresan, however, were tested, chiefly for the purpose of comparing the rate (½ oz. per bu.) recommended in the United States with that (1½ oz. per bu.) recommended in Canada.

Each year, field plans and seed for the entire experiment were prepared at Winnipeg and distributed to the various cooperators. The seed was put up in paper coin-envelopes (one envelope for each row) at the rate of 300 seeds per envelope for seed germination tests, and in lots equivalent

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FIG. 1. Kinds of seed used in cooperative seed-treatment experiment. A. Sound seed. B. Fractured seed.

to a seeding rate of 28 lb. per acre for yield trials. Before the seed was dispatched to the cooperators, envelopes containing seed treated with the same preparation were tied together and wrapped with wax paper to reduce to a minimum the escape of volatile constituents present in some of the preparations.

A uniform, randomized, split-block plan of experimentation (4 replicates) was used at all stations. One-half of each block was sown with sound seed and the other half with fractured seed. The different treatments were randomized within each half-block. In the seed germination

TABLE 1.—*Location of tests in the cooperative seed treatment investigation with flax*

State or Province	Location of test	Cooperators
New York	Geneva	W. F. Crosier
Iowa	Ames	C. S. Reddy
Wisconsin	Madison	H. L. Shands, D. C. Arny
Minnesota	St. Paul	M. B. Moore
South Dakota	Brookings	W. F. Buchholtz, C. N. Nagel
North Dakota	Fargo	W. E. Brentzel
Montana	Bozeman	R. H. Bamberg
Washington	Pullman	C. S. Holton
Manitoba	Winnipeg	F. J. Greaney, J. E. Machacek
	Morden	W. R. Leslie, W. J. Breakey
	Brandon	M. J. Tinline, W. H. Johnston
	Gilbert Plains	A. H. Parker
Saskatchewan	Indian Head	J. G. Davidson
	Melfort	M. J. McPhail, W. T. Burns
	Scott	A. G. Kusch
	Swift Current	A. W. Platt, B. C. Jenkins
	Saskatoon	H. W. Mead
Alberta	Lethbridge	W. D. Hay
	Lacombe	F. H. Reed, G. E. Delong
	Beaverlodge	W. D. Albright, E. C. Stacey
	Edmonton	A. W. Henry

tests, carried on in Canada and the United States, individual plots consisted of single rows, 10 ft. long and 1 ft. apart. In the yield trials, all of which were in Canada, the plots consisted of 4 or 5 rows, 18 ft. long and 9 in. apart. The 5-row plots were used at a few stations where it was considered advisable to obtain information on both germination and yield, one row of the plot being pulled to obtain a germination count for the plot. To furnish information on yield, the middle 2 rows of the remaining 4 rows in such plots were harvested, after 9 in. from the ends of each row had been discarded. In yield trials where 4-row plots were employed, the same general harvesting procedure was followed.

Each year, the experimental data obtained at cooperating stations away from Winnipeg were forwarded to the authors for compilation and analysis. A report on the whole project was subsequently dispatched from Winnipeg to each cooperator. Also, in 1944 and 1945, interim reports were published in the Plant Disease Reporter.[4, 5]

EFFECT OF SEED FRACTURING ON GERMINATION AND YIELD

It was shown earlier[6] that the percentage germination of fractured flax seed is generally lower than that of sound seed, and that the decrease in germination is roughly proportional to the percentage of fractured kernels present in the seed. It was shown, too, that seed fracturing, unless a high percentage of seeds is affected, does not always adversely affect the yield. It appeared from observations on growth that the increased tillering of flax plants in thin stands tended to compensate for loss of seedlings resulting from decay of fractured seed in the soil. In the present experiment, conducted over a wider area, a somewhat similar result was obtained. Although the percentage germination of fractured flax seed was decidedly lower than that obtained with sound seed at most of the cooperating stations, at only a few did seed-fracturing adversely affect the yield.

EFFECT OF VARIOUS SEED TREATMENTS ON GERMINATION

The data summarized in table 2 show that, in 1944 and 1945, none of the seed treatments had any appreciable effect on the germination of sound seed. In 1946, however, some of the treatments increased the germination of such seed by a significant amount—a fact indicating the possible presence of pathogenic organisms on the seed.

Fractured seed responded favorably to seed treatment in each year of the 3-year experimental period. In 1944, a significant increase in germination was obtained for all the seed treatments employed with the exception of New Improved Ceresan applied at the low rate of ¼ oz. per bu. In 1945, significant increases in germination were obtained for all the treat-

[4] Greaney, F. J. Co-operative flax seed treatment tests in 1944. U. S. Dept. Agr., Plant Dis. Reptr. Suppl. 159: 215–219. 1945.

[5] ———. Co-operative flax seed treatment tests in 1945. U. S. Dept. Agr., Plant Dis. Reptr. 30: 113–119. 1946.

[6] Machacek, J. E. and A. M. Brown. Threshing-injury to flax seed in Canada. Scient. Agr. 25: 601–625. 1945.

TABLE 2.—*Effect of various seed treatments on the percentage germination of sound and fractured Royal flax seed*

Treatment	Dose (oz./bu.)	Sound seed[a]			Fractured seed[b]		
		1944[c]	1945	1946	1944[c]	1945	1946
New Improved Ceresan	0.25	61.3	65.5	58.6	28.5	32.2	35.3
	0.50	62.0	67.0	60.6	29.4	36.7	41.0
	1.00	60.0	66.8	63.0	33.9	41.2	49.0
	1.50	60.5	66.8	61.3	33.4	45.4	51.0
	2.00	61.6	66.4	64.3	36.4	46.9	49.6
	3.00	60.9	.....	.....	35.3	.....	.....
Semesan Jr.	2.50	60.0	.....	.....	30.9	.....	.....
Dubay 1452F	0.50	.....	66.5	61.0	.....	34.5	40.6
	1.00	.....	65.8	62.6	.....	37.1	43.0
Spergon	1.50	58.4	65.8	54.6	29.5	31.3	38.0
	3.00	60.0	65.5	58.3	30.2	33.8	36.3
Arasan	1.50	60.8	65.9	60.0	29.3	36.5	44.3
	3.00	60.0	66.0	59.0	32.0	39.8	39.6
Control	.....	60.0	65.2	54.6	25.1	30.4	33.0
Significant difference (5 per cent level)		D.N.S.[d]	D.N.S.	3.5	3.9	2.5	5.1

[a] Threshed with ordinary clothes wringer. There were no fractured kernels present in the seed used in 1944 and 1945. In 1946, four per cent of the kernels were fractured.

[b] Threshed with stationary thresher. Content of fractured kernels was 31 per cent in 1944, 48 per cent in 1945, and 53 per cent in 1946.

[c] Mean of data from 11 stations in 1944 and 13 stations in 1945 and 1946.

[d] D.N.S. = Difference not significant.

TABLE 3.—*Effect of various seed treatments on the yield of sound and fractured flax seed*

Treatment	Dose (oz./bu.)	Yield (bu./acre)					
		Sound seed[a]			Fractured seed[a]		
		1944[b]	1945	1946	1944[b]	1945	1946
New Improved Ceresan	0.25	22.2	28.4	22.5	22.2	22.3	19.2
	0.50	21.0	27.6	21.4	22.0	23.6	20.4
	1.00	22.4	27.7	21.1	23.2	26.0	21.1
	1.50	22.5	28.6	21.5	22.0	27.2	21.4
	2.00	23.1	28.5	23.4	23.0	26.9	21.5
	3.00	22.4	.....	.....	21.2	.....	.....
Semesan Jr.	2.50	21.6	.....	.....	21.5	.....	.....
Dubay 1452F	0.50	.....	28.3	21.7	.....	25.4	20.1
	1.00	.....	27.9	22.6	.....	24.9	20.8
Spergon	1.50	20.6	27.9	22.0	21.2	21.6	19.5
	3.00	22.0	.....	.....	22.5	.....	.....
Arasan	1.50	22.0	27.2	21.9	21.2	24.0	20.3
	3.00	22.9	.....	.....	23.0	.....	.....
Control	.....	21.5	28.4	21.7	21.2	20.6	17.9
Significant difference (5 per cent level)		D.N.S.[c]	D.N.S.	D.N.S.	D.N.S.	2.3	2.1

[a] For percentages of fractured kernels in seed, see footnotes under table 2.

[b] Mean of data from two stations in 1944, four stations in 1945, and 10 stations in 1946.

[c] D.N.S. = Difference not significant.

ments tested except New Improved Ceresan (¼ oz. per bu.) and Spergon (1½ oz. per bu.). In 1946, the results were about the same as in 1945 except that Spergon did not improve germination even when used at the rate of 3 oz. per bu. At some stations (Pullman, Washington; Fargo, North Dakota; and Geneva, New York) the response to seed treatment was usually very slight.

EFFECT OF VARIOUS SEED TREATMENTS ON YIELD

None of the treatments applied to sound flax seed increased the yield, even in 1946 when germination of sound seed was improved considerably by seed treatment (Table 3). With moderately-fractured seed, such as was used in 1944, the observed increases in yield were not statistically significant, but with somewhat more severely-fractured seed, such as was used in 1945 and 1946, increases were significant. These increases were relatively large in 1945, but comparatively small in 1946. There was a good deal of variation in the amount of benefit from treatment, but unlike the situation observed with seed germination, there was no station at which seed treatment continually appeared to be without worth.

From table 3, it would appear that New Improved Ceresan, when applied to fractured flax seed at the rate of 1½ oz. to 2 oz. per bu. increased the yield to the greatest extent. Only in 1945, however, was the yield at these dosages significantly higher than that obtained with the dosage (½ oz. per bu.) generally recommended in the United States, while in no instance was it significantly higher than the yield from the dosage (1 oz. per bu.) generally recommended for the drier parts of Western Canada. Dubay 1452F and Arasan also significantly increased the yield but not so much as New Improved Ceresan. As only one or two dosages of these treatments were employed it was impossible to determine if they were the optimal ones. Treatment of flax seed with Spergon did not bring about any increase in yield.

SUMMARY AND CONCLUSIONS

Cooperative tests of various seed treatments, applied to sound and fractured flax seed, were carried on during a 3-year period over a wide area of Western Canada and in the northern part of the United States. In all, 13 stations in Canada and 8 stations in the United States participated.

The data obtained from the experiment indicate that treatment of flax seed, particularly when a considerable proportion of the seeds are fractured, is a sound practice. It was found that treatment of seed damaged by fracturing generally increased germination and occasionally increased yields. The data suggest also that, when New Improved Ceresan is used, it should be applied to flax seed at a higher dosage than ½ oz. per bu. An increase of this dosage to at least 1 oz. per bu. is indicated.

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NONSUSCEPTIBLE HOSTS AS CARRIERS OF WILT FUSARIA[1]

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INTRODUCTION

Since 1932, stems of many thousands of plants have been cultured on agar plates to isolate wilt Fusaria. At first the cotton plant chiefly was used, but in recent years, a variety of plants has been included, especially in the numerous inoculation experiments. In a study of the pathogenicity and cultural characteristics of the cotton-wilt organism, Armstrong *et al.* (3) reported that a *Fusarium* could be obtained from a fairly high percentage of inoculated cotton plants of a resistant variety that showed no discoloration of the internal tissues. It was assumed that most of the Fusaria recovered were the cotton-wilt fungus, and limited tests indicated that this was generally true. When inoculations of various species of plants were made in considerable numbers, as for example the sweet potato inoculated with the cotton-wilt fungus, Fusaria were likewise obtained from a fairly high percentage of the stalks when cultured on agar plates, although either no or slight external or internal symptoms of wilt were apparent. These results led to the assumption that some of the wilt Fusaria, supposedly restricted in their host ranges, are parasitic[3] on many plants that show no symptoms of disease. Experimental data to support this assumption are presented.

METHODS

Plants were grown in soil, in solution-culture, and in steamed sand. The inoculation procedures in soil using a wheat-oats mixture (3) and in solution-culture (1) have been described. For the steamed-sand cultures, the plants were grown in 2-gal. glazed pots with a 2-cm. hole laterally at the bottom for drainage and either of two inoculation procedures was used. In Method A the roots were inoculated by pouring a 3-day-old solution culture of the fungus into holes made around the base of the plants growing

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[3] Definitions submitted by the Committee on Technical Words. Phytopath. 30: 361–368. 1940. RESISTANCE: "Ability of the host to suppress or retard the activity of a pathogenic organism or virus." NONSUSCEPTIBLE is used in preference to resistant since there are, for example, varieties of cotton which are resistant to the cotton-wilt Fusarium, but cotton is considered nonsusceptible to the sweet-potato-wilt Fusarium. PARASITE: "Organism or virus for which the tissues of another living organism serve as substratum and source of nutrition." The organisms dealt with in this paper are considered parasitic but not pathogenic since there were "either no or slight external or internal symptoms of wilt" when the plants were inoculated with them.

in the pot. In Method B, the roots of young plants which had been grown in flats of sterilized soil were washed, dipped in the inoculum, and set immediately in a pot of sand. In both methods a second inoculation was made a week or more later using the procedure in Method A if the plants showed no symptoms of wilt at that time. Method A is essentially that of Tharp,[4] and Method B is a modification of that described by Wellman (12).

All methods have given approximately the same results. With the solution-culture technique somewhat more care is necessary to prevent contaminations, although a greater number of plants can be grown in a given area by this method than in soil or sand. The procedures for preparing and sterilizing the wheat-oats mixture and incorporating it in the soil are time-consuming, and serious difficulties may be encountered with damping-off due to the addition of considerable organic matter unless the soil is kept for two or three weeks before planting. Certain soils become toxic to plants after steaming which was particularly true of the manganiferous Lloyd soil used in this investigation. The sand-culture technique was very satisfactory, and Method A was used wherever possible. Check plants and those not infected upon inoculation grew vigorously notwithstanding the cutting of some roots.

In Methods A and B of the sand-culture technique the inoculum was prepared by culturing the fungus for 72 hr. at 28° C. on the following liquid medium in a flask which was shaken thoroughly three or four times daily to keep the mycelium well dispersed: sucrose or glucose, 2 per cent; $MgSO_4$, 0.003 M; KCl, 0.022 M; KH_2PO_4, 0.1008 M; $Ca(NO_3)_2$, 0.10356 M; $FeCl_3$, $MnSO_4$, and $ZnSO_4$, 0.2 p.p.m. each of cations. The isolates of Fusarium were maintained on slants of potato-dextrose agar. A suspension of the fungus for inoculating the liquid medium was made by pouring 10 ml. of sterile water on a fresh slant-culture, loosening spores and mycelium with a sterile needle, and using this at the rate of 2 ml. per 1000 ml. of liquid medium.

The nutrient solution described by Armstrong (1) was used for growing the plants. Good growth was obtained with all plants except sweet potatoes which required increased iron up to double that indicated.

During the course of the experiments, the usual precautions were taken to prevent accidental contaminations. Sterilized rubber gloves were worn when making the inoculations for each isolate, and all the necessary glassware and instruments were also sterilized.

Cotton, Cassia (*Cassia tora* L.), okra, soybean, and cowpea were grown in pots of sand and Method A was used in the inoculation experiments. Immediately before planting, the seeds of cotton, Cassia, and okra were immersed in concentrated sulphuric acid for 5 min. and then thoroughly washed in tap water. Seeds of soybean and cowpea were surface sterilized in a hypochlorite solution. Twenty seeds were planted per pot evenly spaced in a circle 1 inch from the periphery and ¾ inch deep. Tap water

[4] Personal communication from Dr. W. H. Tharp, Jr.

was used sparingly until all seedlings had emerged. After about seven or eight days when the plants were usually thinned to ten per pot, nutrient solution was used. The first inoculation was made when the unexpanded first true leaves appeared which was about 10–14 days after planting for cotton but somewhat sooner for the others, depending upon light conditions and temperatures. The second inoculation was made one week later. The inoculation procedure was as follows: 1 hr. after applying the nutrient solution, an inverted Buchner funnel was pressed into the moist sand to a depth of about 1½ inches. Five hundred ml. of the well shaken inoculum was poured into the depression where the roots had been cut. The roots were then covered and after a few hours about 1 quart of tap water was applied to each pot. The same procedure was used for the second inoculation a week later except that when a large number of different isolates was involved, it was more convenient to use a large sterile test tube instead of a funnel for cutting the roots in each pot. Dr. Tharp used 1 qt. of nutrient solution twice daily when the plants began to transpire rapidly but this amount was used only once daily in these experiments with satisfactory results. Large plants, however, frequently required water in addition to the nutrient solution to maintain their turgidity. Once a week tap water was used in place of the solution. The tests with cotton were continued for six to seven weeks, and those with the other hosts for eight weeks.

Method B was used in the experiments with seedlings of tomato, sage, tobacco, snapdragon, mimosa (*Albizzia julibrissin* Duraz), and Mexican clover (*Richardia scabra* St. Hil.). For sweet potato, sprouts or vine cuttings which had been rooted in sand were used, and, if a clear-cut injury was not evident at the base, a small portion of the stem was cut with sterilized scissors before the roots were dipped in the inoculum. Experience has shown that a low percentage of wilt occurs in plants without an injured basal stem. Poole and Woodside (8) stated that infection is almost entirely through the basal end of the plant. After the roots were dipped in a 3-day-old liquid culture of the fungus, five plants were set per pot of sterilized sand and 500 ml. of the inoculum distributed around them. Only 250 ml. was used for sweet potato since a larger amount seemed at times to be somewhat toxic to the tender sprouts and vine cuttings. However 500 ml. of inoculum was applied in the second inoculation. The second inoculation, when necessary, was made one week or more later, depending upon the condition of the plants, by cutting some of the roots with a large test tube placed close to the stem of each plant and distributing the inoculum among the holes made by the tube. At times such definite symptoms of wilt appeared after the first inoculation that a second was clearly unnecessary. The sweet-potato plants showed either very definite or no external wilt symptoms in a relatively short time, consequently, in the later tests they were not grown so long as the other plants but were removed for plating at about four weeks.

Plants in all of the experiments were removed from trays or pots when

definite external symptoms of wilt were apparent. A piece of the basal stem was washed in running water, after which a 1-inch section just above the soil line was cut, dipped in 95 per cent ethyl alcohol, immersed for 5 min. in a calcium hypochlorite solution (BK)[5] containing 1667 p.p.m. of available chlorine, and plated on 1.8 per cent water agar. This concentration of hypochlorite was used on all types of tissue and the procedure was standard throughout these experiments. Plants which had not wilted at the end of an experiment were usually treated similarly.

Efficacy of Hypochlorite as a Surface Disinfectant

The assumption has been that the wilt Fusaria recovered from the stems of plants which showed no wilting were actually inside the stems. If the Fusaria were only attached to the outside surfaces and the hypochlorite failed to kill the organisms, the results as reported would have no significance. Even though the stems did not contain a wilt *Fusarium*, other fungi frequently grew from the pieces. It was important to know whether the hypochlorite disinfects the stem surfaces and, particularly, if it kills the wilt Fusaria which might adhere to the base of the stems. The efficacy of the hypochlorite as a surface disinfectant in the concentration used throughout this work, viz., 1667 p.p.m. of available chlorine, was checked by dipping 24 1-inch, thin, wooden sticks in each of two suspensions of different Fusaria. The sticks were surface sterilized and plated according to the standard procedure described for the stem sections. The suspensions of a sweet-potato-wilt and a cotton-wilt *Fusarium*, were made by adding 10 ml. of sterile water to a test-tube slant and either scraping the surface with a needle or shaking the tube in which the cotton plug was replaced by a sterile rubber stopper. The scraping methods loosened pieces of agar which had to be removed by straining through a thin layer of cotton, before complete sterilization was obtained. An examination of both of the suspensions showed that pieces of mycelium and the three spore forms were present in abundance. A check consisted of ten sticks dipped in each of the suspensions, with sterile water substituted in the subsequent steps for the alcohol and the hypochlorite, to ascertain if the spores and the mycelia had been washed off in the dipping process. All of the sticks that had been treated with hypochlorite were sterile after incubation at 28° C. for two weeks. In a few days, the Fusaria grew abundantly on 18 sticks of the check or unsterilized series. These results indicate that the hypochlorite in the concentration used in these experiments will kill the wilt Fusaria on ordinary surfaces.

EXPERIMENTAL RESULTS

Inoculations of Sweet Potatoes with a Cotton-Wilt Isolate and with Other Fusarium Isolates from Healthy Sweet Potatoes

In the summer of 1940, sweet potatoes growing in a solution culture in the greenhouse were inoculated with the cotton-wilt fungus. Both the

[5] The commercial product, BK, was used.

Nancy Hall and Porto Rico varieties were grown in the first experiments, but since no differences in the susceptibility of the two were noted, thereafter only the Porto Rico variety was used. The plants showed no external symptoms of wilt but a *Fusarium* similar to the original in cultural characteristics was obtained from 70.3 per cent of the plants when stem sections were plated (Table 1, Exp. 1, a). Since the bases of the stems in the check or uninoculated tray (Exp. 1, b) showed no Fusaria when cultured on agar plates, it was inferred that the sweet potato might be a carrier of the cotton-wilt fungus and thus show no symptoms of wilt. It is generally assumed that many of the wilt Fusaria will survive in the soil for long periods without the presence of the usual host plants. If they occur as benign invaders in hosts other than those considered susceptible, another method of their survival will have been revealed.

In other experiments in which sweet-potato plants were inoculated with various wilt organisms, Fusaria were repeatedly recovered from some of the healthy plants in the check trays or pots (Table 1, Exp. 2, a–b; 4, b) as well as from some of the plants which had been inoculated. Thus it seemed that plants produced from bedded potato roots only rarely were fusarium-free, although they were healthy and showed no external symptoms of wilt.

During the course of these experiments, uninoculated plants of cotton, okra, tobacco, tomato, soybean, cowpea, two weeds, and snapdragon were being grown in solution cultures or in steamed soil or sand. Stem sections of many of these were plated, but *Fusarium oxysporum* Schl. according to the system of Snyder and Hansen (11) was seldom observed. Thus it appeared that the technique used was not responsible for the infection of the uninoculated sweet-potato plants but that unblemished sweet potatoes might contain Fusaria which grew into the sprouts when the potatoes were bedded. The sweet-potato wilt pathogen, *F. oxysporum* f. *batatas* (Wr.) S. and H. (5) is known to pass from the bedded root into the sprouts. It was necessary, therefore, to study more carefully the Fusaria occurring in the healthy roots and sprouts so that the results obtained in the pathogenicity tests could be evaluated. Accordingly, certified sweet potatoes were obtained and 20 of these were treated in a hypochlorite solution for 10 min. Under aseptic conditions in a culture-room, pieces of internal tissue from near the stem end, the basal end, and the middle of each potato were plated on water agar. A Fusarium isolate was obtained from only two potatoes and these isolates were used separately to inoculate plants in trays of solution culture in order to determine their relationship to the sweet-potato-wilt fungus. The combined results are given in table 1, experiment 4, a. Likewise comparable trays of plants (Table 1, Exp. 3) were inoculated, respectively, with four isolates from the healthy check plants (Table 1, Exp. 2, a–b). A tray of uninoculated plants (Table 1, Exp. 4, b) was included. The plants for these tests were obtained from certified potatoes which had been surface disinfected before being bedded in the greenhouse in steamed sand in a disinfected bench.

There were no external wilt symptoms in any plant, though some slight internal discoloration was noted in from 12.5 to 22.8 per cent of the plants. This may not be significant, however, since Fusaria were recovered from two to three times as many plants as those which showed internal discoloration viz., from 39.6 per cent of the check plants and from 54 per cent of one of the inoculated groups. Various investigators have noted darkening of the vascular bundles of the stems of plants affected with Fusarium wilts and many have used it as a criterion of infection. Cook (4) has shown, however, that browning of the bundles of tomato stems is an inadequate criterion of infection.

The isolates used for these inoculations were grown on rice, Irish-potato plugs, and potato-dextrose agar along with some wilt pathogens from sweet potato, cotton, and tobacco. The microscopic and cultural characteristics of the isolates indicated that all belong to the section Elegans as described by Wollenweber and Reinking (13).

The results obtained in the previous experiments, especially from plating sections of both the healthy potatoes and the plants produced from the sprouts of these potatoes, seemed to indicate more clearly that sweet potatoes harbor Fusaria internally which are not the sweet-potato-wilt pathogen. It should be noted that Fusaria were obtained from 10 per cent (2 of 20) of the potatoes but from 39.6 per cent (Table 1, Exp. 4, b) of the plants grown from them. One would not expect too close a correlation of such results since only three small pieces of each potato were cultured. The entire potato is bedded and there is a tendency for the sprouts to be produced in groups at the ends.

Since a further study of a larger number of potatoes was desirable, three lots of potatoes were secured, one each from Spartanburg, S. C., from Clemson, S. C., and from the Edisto Experiment Station, Blackville, S. C. Seventy potatoes from each lot were immersed in hypochlorite solution for 6 min., and, following the procedures previously described, a piece of the internal tissue about an inch from each end of every potato was plated on water agar. Fusaria were obtained from 14.3, 7, and 7 per cent, respectively, of the three lots. A discoloration of the vascular ring of some of the potatoes, varying from fairly distinct to slight, was noted but no correlation existed between this condition and the isolation of Fusaria from the potatoes. For example, in the Spartanburg lot, Fusaria were obtained from only five of the 20 potatoes showing discoloration, however, five other potatoes appearing perfectly normal also contained Fusaria.

The potatoes of the three lots from which Fusaria were not obtained were bedded separately by lots in clean beds of steamed sand in the greenhouse. Those from which Fusaria were obtained were bedded similarly. Sprouts from the former were considered as fusarium-free, while those from the latter were considered as possibly diseased. Sprouts from the various lots were grown separately in trays of solution culture, but the results have been combined in table 1, experiments 5, a–b. Even if some of the

original potato roots harbored Fusaria and some apparently did not, the plants from both sources contained a rather high percentage of Fusaria without showing symptoms of wilt.

The plants in table 1, experiment 6, were inoculated with a *Fusarium* obtained from one of the healthy Edisto potatoes, but no wilting occurred.

Since the cotton-wilt organism is present in some of the fields of the Edisto Experiment Station where sweet potatoes are grown it was thought that some of the Fusaria obtained from healthy potatoes might be the cotton-wilt fungus. Accordingly, the isolates from the healthy Edisto potato (Table 1, Exp. 6) was used to inoculate two trays containing 173 cotton plants of a wilt-susceptible variety but no wilting occurred.

TABLE 1.—*Results of inoculations of sweet potato with a cotton-wilt Fusarium and with other Fusaria obtained from healthy sweet potatoes*

Exp. No.	Origin of isolate	No. Plants	Medium for growing plants	Percentage of plants with symptoms of wilt	Recovery of fungus, per cent
				Internal discoloration[e]	
1a	Cotton	37	Solution	24.3	70.3
b	Uninoculated check	30	do	0.0	0.0
2a	do	148	do	6.8	55.4
b	do	10	Soil	0.0	20.0
3	Healthy potato plants[a]	197	Solution	22.8	47.2
4a	Healthy potato roots[b]	100	do	18.0	54.0
b	Uninoculated check	48	do	12.5	39.6
5a	Uninoculated[c]	107	do	12.1	65.4
b	Uninoculated[d]	87	do	36.7	62.1
6	Healthy potato roots	145	do	2.1	60.7

[a] Four trays, each with a different isolate.
[b] Two trays, each with a different isolate.
[c] Potatoes which showed no fungus on plated tissues; sprouts from these grown without inoculation.
[d] Potatoes which showed a Fusarium on plated tissues; sprouts from these grown without inoculation.
[e] There were no external symptoms of wilt in any of the plants.

Recovery of the Cotton-Wilt Fusarium from Sweet Potato and Snapdragon

After numerous observations indicated that wilt Fusaria invaded non-susceptible hosts, an attempt was made to isolate some of the Fusaria from these hosts and, by inoculations into a susceptible one, to determine whether the wilt organism had been recovered. Therefore, ten sweet-potato plants were grown in two pots of sand and inoculated with the cotton-wilt fungus. No symptoms of wilt were evident but Fusaria were recovered from nine of the ten plants. An isolate from each plant was used to inoculate a pot of cotton, and, as noted in table 2, experiment 1, eight of these proved to be the cotton-wilt fungus. The ninth isolate was not included in the data because it was nonpathogenic to cotton.

Since the cotton-wilt fungus can invade sweet potatoes when inoculations are performed under greenhouse conditions, it was of interest to know if

natural invasion occurs in the field. Disease-free sweet potatoes were bedded in steamed sand and both sprouts and vine cuttings were set in a field where cotton had died of wilt. Three settings were made from July 24 to August 1, 1944. Sprouts and vine cuttings were also planted in steamed sand in the greenhouse to check if any wilt might appear but none occurred in either the greenhouse or the field. On October 10, sixty-eight plants were brought to the laboratory for plating and Fusaria were obtained from 61 of these. Some of the isolates did not belong to *Fusarium oxysporum*, so only those which appeared most promising from 46 of the plants were tested for pathogenicity on cotton growing in pots of sand. When the isolates were tested singly or in combinations of two, the results showed that at least 26 sweet-potato plants carried pathogenic isolates of the cotton-wilt fungus, although there may have been as many as 34 since this was the number involved in the various combinations. The percentage of cotton plants wilting in the individual pots was in the range from 81.8 to 100 per cent with an

TABLE 2.—*Results of inoculations of cotton with the cotton-wilt Fusarium recovered from inoculated sweet potatoes or those naturally invaded in the field, and from inoculated snapdragons*

Exp. No.	Origin of isolate	No. cotton plants inoculated	Percentage wilt	Medium for growing plants
1	Sweet potato[a]	94	97.9	Sand
2	Sweet potato, field[b]	338	95.6	do
3	Sweet potato, field[c]	114	0	do
4	Snapdragon	11	91.7	do

[a] Eight isolates from 10 plants tested singly. Ninth not included was nonpathogenic.
[b] Fifty-three isolates from 34 plants tested singly or in combinations of 2 in 27 pots.
[c] Seventeen isolates from 12 plants tested singly or in combinations of 2 in 10 pots.

average of 95.6 per cent for the series (Table 2, Exp. 2). The isolates from 12 of the sweet-potato plants proved nonpathogenic to cotton (Table 2, Exp. 3). These results indicated that the cotton-wilt fungus can invade sweet-potato plants in the field as well as by inoculations in the greenhouse, so there appears to be nothing particularly artificial in the results obtained in the greenhouse.

Twelve snapdragon plants in solution culture were inoculated with the cotton-wilt fungus. No wilting occurred but a *Fusarium* was isolated from two of the plants. The isolates were mixed for the inoculation of cotton and 91.7 per cent of the plants of the latter wilted as shown in table 2, experiment 4.

Recovery of the Tomato-Wilt Fusarium from Sweet Potatoes and Mimosa

Ten sweet-potato plants growing in sand were inoculated with a tomato-wilt *Fusarium* but showed no symptoms of wilt in eight weeks. Fusaria were obtained from all plants, but only the isolates from eight were used in four pairs to inoculate a total of 20 Pennred tomato plants growing in pots of sand. Since all the tomato plants wilted, it is evident that the tomato-wilt *Fusarium* was recovered from the sweet potatoes.

One of the 13 mimosa plants inoculated with a tomato-wilt *Fusarium* showed a dark streak which extended 3 inches upward in the basal region of the stem although the plant was vigorous and showed no external symptoms of disease. Two Fusarium isolates obtained from this stem were pathogenic to tomato.

Recovery of the Sweet Potato-Wilt Fusarium from Cotton

Fifty-six cotton plants of the Rowden variety growing in a tray of culture solution were inoculated with a sweet-potato-wilt isolate. There were no external symptoms of wilt but Fusaria were recovered from 12.5 per cent of the stems. Three of the recovered isolates when used to inoculate sweet-

TABLE 3.—*Results of inoculations of sweet potato with the sweet-potato-wilt Fusarium recovered from cotton which was inoculated in the greenhouse or naturally invaded in the field*

Exp. No.	Sweet-potato isolate recovered from	No. sweet-potato plants inoculated	Percentage wilt	Medium for growing plants
1a	Cotton[a]	117	100	Solution
b	Cotton[b]	30	100	Sand
2a	Cotton, Resistant, field[c]	40	100	do
b	Cotton, Resistant, field[d]	45	0	do
c	Cotton, Susceptible, field[e]	70	97.1	do
d	Cotton, Susceptible, field[f]	55	0	do
3a	Cotton	5	0	do
b	Cotton[g]	20	100	do
c	Cotton[h]	20	100	do
d	Cotton[i]	20	100	do

[a] Three isolates, each used separately.
[b] Same isolates as a, used separately in sand.
[c] Eleven isolates used singly or in combination in 8 pots.
[d] Thirteen isolates used singly or in combination in 9 pots.
[e] Twenty-one isolates used singly or in combination in 14 pots.
[f] Thirteen isolates used singly or in combination in 14 pots.
[g] Four isolates from wood in basal inch of stem, each used separately.
[h] Four isolates from bark in basal inch of stem, each used separately.
[i] Four isolates from wood of tap root, each used separately.

potato plants grown in solution culture and in sand caused all plants to wilt (Table 3, Exp. 1, a–b). This was interpreted as evidence that the sweet-potato-wilt fungus can penetrate the cotton stem and exist there without producing wilt. To ascertain if this fungus will invade cotton in the field, seed of the wilt resistant variety 4-in-1, and the susceptible variety Rowden, were planted in a field where sweet-potato plants had died of wilt. Eighteen days after planting, 32 and 34 seedlings, respectively, of the resistant and susceptible varieties were pulled, and a short basal section of each stem was plated on water agar, using the method previously described. Seventeen isolates of *Fusarium* were obtained from the Rowden plants and 20 from the 4-in-1 plants. Sixty-three days after planting, the remaining plants were pulled and treated as above. None of the plants showed any external symptoms of wilt yet 18 Fusaria were obtained from 59 of the 4-in-1 plants,

and 25 from the 61 Rowden plants. No effort was made to identify all of the Fusaria obtained but only the cultures which appeared to be *Fusarium oxysporum* were used in pathogenicity tests.

The difficulties of making pathogenicity tests with all of the isolates were somewhat reduced by testing some of them in combinations, as is shown in table 3, experiment 2, a–d. At least 8 and possibly 11 of the isolates from the resistant 4-in-1 cotton proved to be pathogenic to sweet-potato plants while 13 of the isolates were nonpathogenic. At least 14 and possibly 21, of the isolates from the susceptible Rowden cotton were pathogenic while 13 of the isolates were nonpathogenic. These results indicate that cotton in the field was invaded by the sweet-potato-wilt *Fusarium* present in the soil and that the invasion of the plants in the greenhouse was not dependent upon special conditions.

Recovery of the Sweet-Potato-Wilt Fusarium from Inoculated Cassia, Mexican Clover, Okra, Sage, Tomato, and Soybean

Numerous inoculation experiments with the sweet-potato-wilt *Fusarium* on various hosts have been conducted. In many cases *Fusarium oxysporum* was recovered from the stems of plants which showed no symptoms of wilt. When some of these isolates were tested on sweet-potato plants growing in sand, it was discovered that the original sweet-potato-wilt organism had been recovered. The results of the inoculations of Cassia, Mexican clover, okra, sage, tomato, and soybean, together with the experimental procedures are summarized in table 4. It will be seen that this wilt organism was recovered from all of these hosts since sweet-potato plants wilted when inoculated with some of the isolates.

TABLE 4.—*Inoculation of several species of plants with the sweet-potato-wilt Fusarium and the subsequent recovery of the fungus as shown by tests on sweet potato*

Host inoculated with sweet-potato-wilt Fusarium	Inoculation data			Recovery data		
				Isolates tested on sweet potato plants growing in sand		
	Medium for growing plants	No. plants inoculated and cultured	Percentage recovery of Fusaria	No. isolates tested	No. plants	Percentage wilt
Cassia	Steamed Soil	17	64.7	2	15	100
Mexican Clover	Solution	14	14.3	2	5	100
					5	0
Okra	Sand	11	90.9	9[a]	15	100
Sage	do	9	66.7	1	5	60
Tomato (Bonny Best)	do	12	25 0	1	5	100
Soybean (Yelredo)	do	12	75.0	2[b]	5	100

[a] In combinations of 3.
[b] Two isolates combined.

Isolation of the Sweet-Potato-Wilt Fusarium from Naturally Invaded Mexican Clover

Mexican clover growing in hills with wilted sweet-potato plants was examined and slightly darkened streaks were noted in the lower region of the stems of several plants. Fusarium isolates were obtained from these sections when they were plated. Several of the isolates were nonpathogenic to sweet potato but one proved to be the wilt organism since it killed inoculated sweet-potato plants grown in sand. Thus a weed which showed no external symptoms of wilt was a carrier of the fungus.

In table 4, it will be noted that the sweet-potato-wilt fungus was isolated from two weeds, Cassia and Mexican clover, and four cultivated plants. Since the fungus was recovered from plants of widely divergent families, it seems reasonable to suppose that it might invade plants of other families. All of the plants were exposed to a rather heavy inoculum but this is apparently not a critical factor since the fungus was recovered from both naturally invaded cotton (Table 3) and Mexican clover.

Recovery of the Tobacco-Wilt Fusarium from Inoculated Cassia, Cotton, Okra, Sage, Mexican Clover, Cowpea, and Soybean

A *Fusarium* isolated from flue-cured tobacco (Tobacco isolate No. 1) causes wilt of flue-cured tobacco, Burley tobacco, and sweet potato. In other experiments not related to this study, it had been noted that a *Fusarium* was rather generally recovered from many plants inoculated with this fungus, even though they were healthy and showed no external symptoms of wilt. The apparent entrance of this fungus into a wide range of plants led to its inclusion in these tests in which okra, Cassia, Mexican clover, cotton, cowpea, soybean, and sage were inoculated. Another tobacco-wilt isolate (Md. Mudd) was tested only on soybean.

The recovered Fusaria were tested for pathogenicity on sweet potato rather than tobacco since the former is easier to grow and also wilts quickly, usually in one to two weeks, whereas it may take as many months to get similar results with flue-cured tobacco.

Detailed data are not presented in tabular form due to limitations of space but the tobacco-wilt *Fusarium* No. 1 was recovered from Cassia, cotton, okra, sage, cowpea, soybean, and Mexican clover, since sweet-potato plants wilted when inoculated with the isolates. The "Md. Mudd"-tobacco *Fusarium* was also recovered from soybean.

Location of Wilt Fusaria in Tissues of Plants Without Wilt Symptoms

Nearly all of the nonsusceptible plants from which wilt Fusaria have been obtained showed neither external nor internal symptoms of wilt except for a slight discoloration of the woody tissues in a small number of cases. Since wilt Fusaria readily invade the vascular systems of susceptible hosts and to some extent those of resistant varieties, it might be supposed that they may be found also in the bundle regions of nonsusceptible hosts.

To locate the fungus in the plant the following experiments were performed. The wilt-susceptible Rowden variety of cotton was grown ten plants per pot in ten pots of steamed sand. After inoculation with a sweet-potato-wilt isolate, the plants grew vigorously, without symptoms of wilt and reached an average height of 36.5 inches in eight weeks when the experiment was terminated. Another pot of ten uninoculated plants was used as a check. As the plants were removed, all stems and roots were washed thoroughly and allowed to dry before being cut into sections. Three sections of the stem were used, viz., a 1-inch section of the base at the soil line, B, the 12th inch from the soil line, C, and the last inch at the tip, D. Later the bark was removed from the wood of the basal and middle sections but not from the tip section. A 1-inch section of the tap root about ½ inch below the basal section of the stem was also removed and later the bark was separated from the wood. Approximately 1-inch sections of three of the side roots were used without separation of bark and wood. All of the sections were treated in hypochlorite solution by the standard procedure and plated on water agar. If the three side roots per plant are considered a unit, 880 units of tissue from 110 plants were surface disinfected, plated, and then observed for the growth of organisms. Where bark and wood were separated, the entire stem or root section was dipped in 95 per cent alcohol and immersed in hypochlorite solution for 5 min. It was then placed in a sterile Petri dish with the lid slightly raised while bark and wood were separated with sterile scalpel and tweezers. The bark was transferred directly to water-agar plates. The woody portion, however, was again put in the hypochlorite solution for an additional 5 min. to obviate the possibility that organisms from the bark might have been transferred to the wood. A transfer of a Fusarium isolate was made to agar slants from each of 190 pieces of tissue on the plates and all but one, were similar in cultural characteristics such as color, type of mycelial growth, lack of sclerotia, etc., to the original sweet-potato isolate used for the inoculations. At least one of these typical cultures was obtained from some section of all but one of the inoculated plants. A portion of the data on the recovery of apparently pure cultures of the wilt fungus is shown in table 5. A typical culture was obtained from root sections of 65 plants and from the basal inch of stem of 62 of the same plants. The largest number of cultures was obtained, however, from either the bark or wood of the basal inch of stem of 96 plants. A typical *Fusarium* was recovered from the bark only of 78 and from both bark and wood of 18 of the basal sections of these plants. The extensive histological studies by other workers with numerous wilt pathogens on susceptible and resistant varieties of their host plants have shown the penetration of various host tissues, especially the xylem and cortex. This study of plated tissues indicates that the sweet-potato-wilt pathogen may also penetrate the above mentioned tissues of the nonsusceptible cotton plant. Penetration of the xylem of the nonsusceptible hosts is not established by the procedures which were used, but the definite darkening of the xylem

region in mimosa, Mexican clover, some of the other plants, and sweet-potato roots, indicates that this may be a possibility. On the other hand, the recovery of a typical sweet-potato *Fusarium* from three to four times as many cortical sections as from the woody sections of cotton indicates that most of the invasion of these plants may be cortical.

An additional 48 Fusaria which were similar in appearance to the wilt fungus were observed on the plates but were not transferred to agar slants. *Fusarium moniliforme* Sheld. appeared on 56 pieces. Some of the more common organisms growing in the plates were species of *Trichoderma, Penicillium, Alternaria, Macrosporium,* and bacteria. *Trichoderma* occurred frequently on root sections and was also found twice on wood sections 12 inches up the stem. It will be noted that the sweet-potato-wilt *Fusarium* was not recovered from either bark or wood of any 12th-inch stem section. A single culture of *F. moniliforme* was recovered from the bark of a 12th-inch stem section and no other Fusaria were found at this height in either bark or wood. Forty-four per cent of the 12th-inch stem sections were sterile and bacteria occurred on most of the others. Similar results were obtained with the tip section. With the exception of *F. oxysporum*, the organisms which occurred in the inoculated plants also were found in the check or uninoculated plants. In addition, *F. roseum* (Lk.) S. and H. was found in pieces of bark of three stems and in two pieces of the wood of the tap root of check plants. Rudolph and Harrison (9) found this organism[6] in the seed and in steles of healthy mature cotton stems 6 inches above the soil line.

Thirteen of the Fusarium isolates were tested for pathogenicity on sweet-potato plants growing in pots of sand. Nine of the isolates were from wood sections, five of these from the basal inch of stem and four from the tap root. The other four isolates were from the bark of the basal inch of stem. All but one isolate, which was different culturally from the others, proved pathogenic to sweet potato as is seen in table 3, experiments 3, a–d. These results showed that the sweet-potato-wilt fungus occurred in both bark and wood of cotton plants but that it was not found 12 inches up the stem in a period of eight weeks. The cotton-wilt fungus usually can be found in the tips of inoculated susceptible cotton plants in an equal length of time. Except in this experiment with cotton, no attempt has been made to determine very accurately the percentage of plants of a nonsusceptible host from which one of the wilt Fusaria can be obtained but it seems likely that the recovery from inoculated plants in the greenhouse will be higher on account of the greater inoculum potential. This is indicated by the experiments with cotton in which the sweet-potato-wilt fungus was recovered from 12 to 17 per cent of the plants in the field and approximately 99 per cent from those inoculated in the greenhouse. Not all of the isolates from the latter, which comprised a series of 100 plants, were tested for pathogenicity but the close similarity in cultural characteristics of the untested iso-

[6] *F. scirpi* Lamb. and Fautr. according to Wollenweber and Reinking.

lates to those which were tested seemed to indicate a recovery of approximately 99 per cent. An examination of all the data, however, shows that the latter instance is unusual since the recoveries of Fusaria from even the succulent sweet-potato stems were in the range of about 20 to 70 per cent.

The occurrence of Fusaria inside the cotton seed was not considered in this study and no attempt will be made to review the literature, which is given in the papers by Armstrong *et al.* (2) and Rudolph and Harrison (9). The assumption that *Fusarium oxysporum* f. *vasinfectum* (Atk.) S. and H. found in seed reached them through the vascular system is plausible since it is a vascular pathogen. Rudolph and Harrison (9) found *F. moniliforme*, *F. oxysporum*, and *F. scirpi* (*F. roseum*) in receptacles, placentae, and seed of mature plants, which they assume reached these tissues through the vascular system since these fungi were found in sections of the stele 6 inches above the soil line. In the present investigation the fungi were found in 2-month-old stems but they had not grown very far up the stems. *F. oxysporum* was not found in any 12th-inch section, *F. moniliforme* was in a single bark specimen at this height, and *F. roseum* was in a few bark specimens at the same height. The only wood section in which *F. roseum* occurred was in the tap root.

TABLE 5.—*Recovery of Fusaria from sections of roots and stems of 100 cotton plants inoculated with the sweet-potato-wilt fungus*

Section of plant	No. of plants giving an apparently pure culture of the wilt fungus
All sections	99
All roots	65
B (stem)[a]	62[b]
B, bark or wood[c]	96
C and D	0

[a] Stem sections of the 65 plants in which the fungus was found in the roots.

[b] The fungus was found in the bark only of 48 plants and in both bark and wood of 14 others.

[c] The fungus was found in the bark only of 78 plants and in both bark and wood of 18 others.

Recovery of a Mixed Culture of Cotton- and Sweet-Potato-Wilt Fusaria from Cotton

The question next arose as to whether more than one wilt *Fusarium* might invade a plant at a given time, and result in a mixed culture when isolated. For example, would a wilted cotton plant contain both the cotton- and the sweet-potato-wilt Fusaria if these were present where it was growing?

Rowden cotton plants, growing in two pots of sand with 11 and 13 plants respectively, were given two inoculations according to the standard procedures using 250 ml. each of liquid inoculum of a cotton- and a sweet-potato-wilt *Fusarium*. A total of 12 wilted plants was removed from the pots. Isolations of Fusaria were made from the basal stem sections of all but one

of these. Each section was cultured separately on a water-agar plate and several transfers from each were made to potato-dextrose-agar slants. It was not difficult to obtain cultures free from bacteria and other organisms that occasionally grew from the stem if the transfers were made some distance from the section. Since the cotton- and the sweet-potato-wilt isolates selected for the study showed distinct cultural differences, it was possible to distinguish roughly which was predominantly present. The cotton-wilt isolate produced a blue-green pigment in the agar and formed sclerotia of this color, whereas, the sweet-potato-wilt isolate produced only purple tints and no sclerotia. The mycelial growth was also different in some respects.

The isolations on the agar slants which were made directly from the cotton stems appeared in some cases to contain only the cotton-wilt fungus, in others only the sweet-potato-wilt fungus, and in still others a mixture of the two. This variation in composition was again demonstrated when transfers of these isolates were made to potato-dextrose-agar plates and the cultural characteristics of the resulting colonies were noted.

To obtain further evidence as to the nature of the cultures, pathogenicity tests were also made in which ten to seventeen cotton plants per pot and five sweet-potato sprouts per pot were inoculated at the same time with some of the same inoculum from an original isolate of each of eight wilted cotton plants. The results of the cultural studies and the inoculation experiments are presented in table 6. A study of the plate cultures of these isolations indicated that a mixed culture was being used for all but two of the isolates. Numbers 1 and 6 seemed to be pure cultures of the cotton-wilt and of the sweet-potato-wilt Fusaria, respectively, and pathogenicity tests further indicated that this was true. Three of the cultures caused cotton to wilt but not sweet-potato even though the plates showed a mixture of Fusaria. Two isolations caused all plants of both cotton and sweet potato to wilt.

In many plates distinct sectors were produced which resembled one or the other of the Fusaria. If it had not been known beforehand that a mixed culture was being used, these sectors might have been taken to be variants of the cotton-wilt *Fusarium* which was isolated from the wilted cotton plant. The composition of a few of these sectors is shown in table 7. A transfer was made from the middle of the sector to a potato-dextrose-agar slant, and the resulting culture was used to inoculate cotton and sweet-potato plants. It will be seen that none of these sectors was a pure culture of either organism even though it had the cultural characteristics of one or the other of them. One sector that appeared to be the sweet-potato-wilt *Fusarium* also contained that of cotton and the transfer made from this sector apparently was predominantly the latter since no sweet-potato plants wilted. Another that appeared to be the cotton-wilt *Fusarium* failed to produce cotton wilt but caused one of five sweet-potato plants to wilt.

These studies suggest the necessity of using monosporous isolates in cross-inoculation experiments since in this case both the sweet-potato- and the cotton-wilt organisms occurred in some of the cultures obtained from

TABLE 6.—*Identity of isolates from wilted cotton plants inoculated with both cotton- and sweet-potato-wilt Fusaria as shown by cultural studies and pathogenicity tests.*

Isolation from plant no.	Isolate on agar slant resembled original culture from:	Isolate on agar plates resembled original culture from:	Inoculation data	
			Percentage wilt of cotton	Percentage wilt of sweet potato
1	Cotton	Cotton	100	0
2	Cotton	Mixture	100	0
3	Sweet potato	Mixture	100	100
4	Mixture	Mixture	100	100
5	Mixture	Mixture	100	0
6	Sweet potato	Sweet potato	0	100
7	Mixture	Mixture	100	40
8	Cotton	Mixture	100	0

wilted cotton plants. If one had used for the inoculations the cultures obtained from either uniform colonies or sectors, it would have appeared that a presumably pure cotton-wilt culture also caused wilt of sweet potato.

TABLE 7.—*Identity by pathogenicity tests of sectors produced on potato-dextrose-agar plates by two isolates from wilted cotton plants inoculated with both cotton- and sweet-potato-wilt Fusaria*

Plate Culture No.	Sector resembled original culture from:	Plant inoculated	No. plants	Percentage wilt
1 (Produced 3 sectors)	Cotton	Cotton	10	0
		Sweet potato	5	20
	Sweet potato	Cotton	13	100
		Sweet potato	5	40
	Sweet potato	Cotton	10	100
		Sweet potato	5	0
2 (Produced 2 sectors)	Cotton	Cotton	14	92.9
		Sweet potato	5	100
	Sweet potato	Cotton	15	93.3
		Sweet potato	5	100

DISCUSSION

It has been shown by the senior author and others that wilt Fusaria may be found in the stems of resistant varieties of the usual host plants without either external or internal symptoms of wilt. When numerous Fusaria were recovered from the stems of inoculated plants of genera or species that were not recorded as susceptible, tests were made to ascertain how commonly plants might serve as carriers of many of the wilt Fusaria. The results presented in this paper seem to establish the fact that the wilt Fusaria are parasitic on many plants that show no symptoms of disease. There seems to be no doubt that most of the wilt Fusaria can persist in the soil as saprophytes, but the infection of weeds and cultivated plants which have not been classed as host plants must affect their persistence in soils. The percentage of the Fusaria recovered from some of the plants in both green-

house and field was not always high, but in the most detailed experiment in the greenhouse the sweet-potato-wilt fungus was apparently recovered from 99 per cent of the inoculated cotton plants.

In several instances the senior author has observed that the cotton-wilt fungus persisted in the soil for periods varying up to 12 years without an intervening crop of cotton, yet the incidence of wilt at the end of these periods was high. The impression has been gained from the literature that rotations of three or four years for the control of tomato wilt serve to reduce noticeably the incidence of this disease. Is the cotton-wilt organism better able to survive the competition in the soil than is the tomato-wilt fungus, or does the cotton-wilt fungus enter a greater number of nonsusceptible hosts than does the tomato organism and thus facilitate its survival? The question cannot be answered from the data which are presented.

The cotton-wilt fungus was not recovered from bedded sweet-potato roots in the single instance where a test was made, but some sound sweet potatoes carry *Fusarium oxysporum* internally and one is led to infer that wilt Fusaria other than the sweet-potato pathogen may also be present. The occasional occurrence of a wilt where it had not been found previously might conceivably be explained by the use of some preceding root or tuberous crop that carried the organism to which the latter was not ordinarily considered susceptible, as well as by infected seed. In connection with other experiments not considered in this paper, *Fusarium oxysporum* was present in the internal tissues of sound Irish potatoes. The certified potatoes which were used had been grown in a region where Irish-potato wilt is said to be practically unknown and where the certification authorities have a zero tolerance for this disease.

Harter and Weimer (5) injected the sweet-potato-wilt fungus into stem wounds at the surface of the soil using *Solanum melongena* L., *Lycopersicum esculentum* Mill., and other plants. There were no external symptoms of wilt although in most cases there was a blackening of the fibrovascular bundles for a centimeter or so about the wound, from which the fungus could be recovered. Rudolph and Harrison (9) found *Fusarium moniliforme, F. oxysporum, F. scirpi* (*F. roseum*), and another unidentified *Fusarium* in cotton seed which the organisms were assumed to have reached through the stele since stem sections 6 inches above the soil line yielded the same Fusaria. We have also found the above mentioned organisms in cotton stems as well as *F. solani* (Mart.) App. and Wr. and some Fusaria as yet unidentified. Smith and Shaw (10) inoculated cotton with a wilt isolate from flue-cured tobacco in four successive tests without the occurrence of typical disease symptoms yet the fungus was recovered from the plants. Padwick *et al.* (7) obtained two Fusarium isolates from field-grown sunn-hemp which failed to cause wilt of sunn-hemp but produced a wilt of pigeon-pea. Yet their experiments showed the pigeon-pea- and sunn-hemp-wilt organisms to be highly specific in causing wilt of each host.

Lutman (6), using the Irish potato plant especially but also several other plants for comparison, attempted to establish the filamentous nature

of the irregular lines which simulate cell walls. These filaments were thought to be Actinomycetes but the isolation of an organism was not attained.

SUMMARY

Healthy sweet-potato roots were shown to carry internally Fusaria of the section Elegans which were not the sweet-potato-wilt fungus. Plants from these roots also contained Fusaria and it is assumed that they came from the parent potato.

Cotton, either inoculated in the greenhouse or grown in a field infested with the sweet-potato-wilt *Fusarium*, was invaded by this organism without showing any symptoms of wilt; and, thus, became a carrier of the fungus. Likewise, no external symptoms of wilt were noted in the plants mentioned below.

Cassia, Mexican clover, okra, sage, soybean, and tomato were inoculated with the sweet-potato-wilt *Fusarium* and the fungus was recovered from the stems as shown by subsequent pathogenicity tests on sweet potato.

The sweet-potato-wilt *Fusarium* was recovered from the weed, Mexican clover, which exhibited no external symptoms of wilt, but grew among wilting sweet potatoes in a field.

Tobacco-wilt Fusaria were reisolated from inoculated Cassia, cotton, okra, sage, cowpea, soybean, and Mexican clover.

Sweet-potato plants were invaded by the cotton-wilt *Fusarium*, either from infested soils in the field or inoculations in the greenhouse.

The cotton-wilt *Fusarium* was reisolated from inoculated snapdragons.

The tomato-wilt *Fusarium* was reisolated from inoculated sweet potatoes and mimosa.

Sections of stems and roots of cotton plants inoculated with the sweet-potato-wilt *Fusarium* were plated on agar to determine the location of the fungus in the host. Bark and wood were separated in the root and stem sections. The fungus was recovered from the wood of the tap root and the basal inch of stem, and also from the bark of the basal inch of stem. It was not recovered from the 12th inch of the stem or higher, indicating that penetration up the stem did not proceed very far in eight weeks.

Mixed cultures of the cotton- and the sweet-potato-wilt Fusaria were used to inoculate cotton, and both Fusaria were reisolated from wilting plants. On many of the agar plates containing the reisolations, distinct V-shaped sectors appeared with characteristics of one or the other of the original cultures. If it had not been known that a mixed culture was being used, these sectors might have been considered variants of the cotton-wilt *Fusarium*. The necessity of using monosporous isolates in cross inoculations with Fusaria to determine host relationships is clearly indicated.

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A NEW VIRUS DISEASE OF BLACKBERRY[1]

NORMAN L. HORN

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In 1942 a single strikingly variegated wild blackberry (probably *Rubus allegheniensis*) was observed near Keedysville, Maryland in a raspberry growing district. The symptom pattern (Fig. 1, A) suggested that the plant might be infected with a virus. Cursory examination failed to reveal similarly affected plants within a radius of approximately 200 yards. In 1947 a portion of this plant was removed to the greenhouse for further study.

During this season all blackberry plants within a 200-yard radius were carefully examined for variegational symptoms. No other plants bearing such extensive variegated areas were found. However, ten plants were located in which a few small bright yellow, sharply-defined chlorotic areas (0.3 to 1 cm.) appeared on one or more leaves. In most instances, however, there was only one small chlorotic area on the entire plant.

Three preliminary approach grafts were made to black raspberry plants of the Logan variety to determine the possibility of transmission to this species. Within three to four weeks symptoms appeared on all of the test plants. These consisted of chlorotic patterns similar to those occurring in the blackberry. Two of these raspberry plants were then grafted to healthy wild blackberry plants. In both cases the original pattern was transmitted.

To test transmissibility further, a series of grafts was made in the greenhouse as follows: (1) five healthy blackberry plants (*Rubus allegheniensis* collected near College Park, Maryland) grafted to the diseased blackberry plant; (2) five healthy raspberry plants (hybrid Cumberland × Evans) grafted to the diseased blackberry plant; (3) five healthy blackberry plants grafted to five healthy raspberry plants, which were used as controls. The extent of transmission and date of first symptoms are shown in table 1. Of the ten plants tested, six developed symptoms of the disease in question. Symptoms appeared on five of the plants 30 days after the grafts were made and on one 73 days after grafts were made. Grafts between diseased and test plants in which transmission did not occur were examined and in each case there was a definite organic union.

In every raspberry plant to which the disease was transmitted, first symptoms appeared about two feet distal to the graft. The chlorotic condition then continued to appear progressively toward the growing point. While symptom expression in the blackberry was similar to that in raspberry, in one case chlorotic areas first appeared on a single leaflet immediately below the graft. This was followed by the appearance of chlorosis in adjoining leaves above and below the first infected leaf. In two at-

[1] Scientific publication No. A189, contribution No. 2096 of the Maryland Experiment Station, Division of Plant Pathology.

TABLE 1.—*Graft transmission of a variegation virus from blackberry to blackberry and to raspberry*

Grafts	Plant number	Number of days before symptoms appeared
Healthy raspberry to variegated blackberry	1	30
	2	No symptoms observed[a]
	3	24
	4	29
	5	73
Healthy blackberry to variegated blackberry	1	No symptoms observed[a]
	2	Do
	3	24
	4	Do
	5	25
Controls		
5 Healthy raspberry to 5 healthy blackberry		No symptoms observed[a]

[a] Grafts were made on September 9, 1947 and no symptoms had been observed by December 20, 1947.

tempts the virus was graft transmitted from artificially infected raspberry back to healthy blackberry without alteration of induced symptoms.

The symptom expression varied considerably in individual blackberry and raspberry leaflets, ranging from leaflets having only a few white islands to leaflets which were nearly totally white (Fig. 2). Willison[2] found that leaves of peach seedlings vary greatly in their response to peach blotch with respect to symptom expression. Figure 1 shows the general pattern

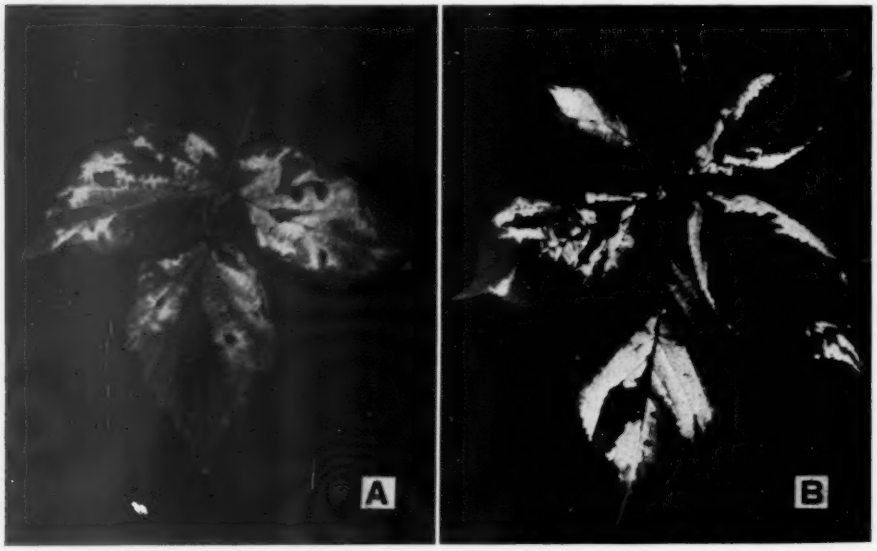

FIG. 1. Typical symptom expression in entire leaves of blackberry and black raspberry. A. Symptoms in blackberry. B. Symptoms in black raspberry.

[2] Willison, R. S. Peach blotch. Phytopath. 36: 273–276. 1946.

of symptom expression in typical raspberry (A) and in blackberry (B) leaves.

Raspberry leaf symptoms are shown in figure 2, (A) through (E). Leaflet (A) shows the occurrence of totally green islands which appear darker green than the normal green areas of the leaf within the chlorotic area. Leaflets (B) through (E) illustrate the various positions of the chlorotic areas. Sometimes these occur along the veins (B), along the leaflet margin (C), as small white islands (D), and as scattered white islands in addition to chlorotic areas along the leaf margin (E). These same symptoms occur in blackberry but certain of the less common chlorotic patterns

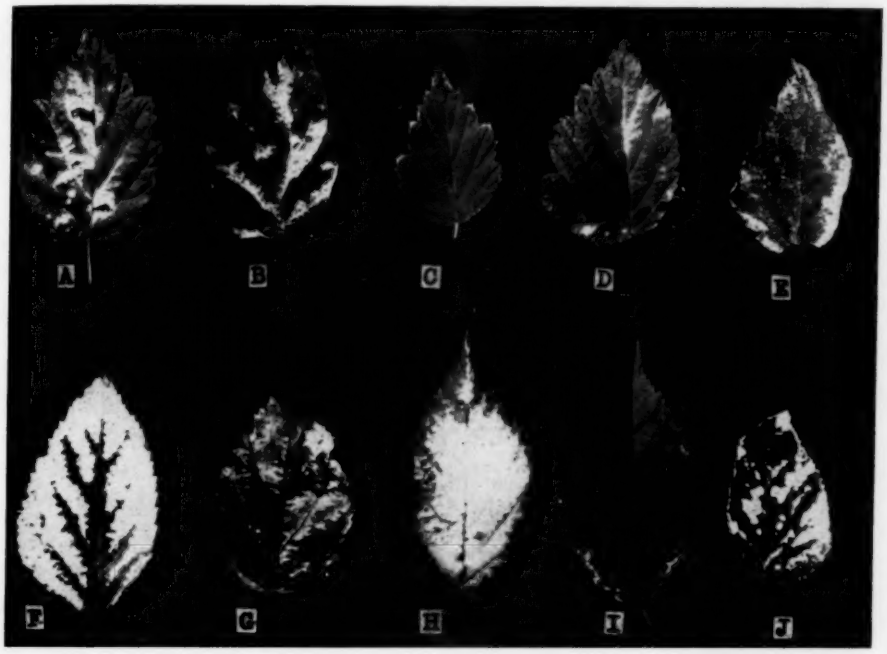

FIG. 2. Symptoms in leaflets artificially infected by grafting with the blackberry variegation virus. A through E. Symptoms in black raspberry. F through J. Symptoms in blackberry.

(Fig. 2. F, H, and J) occur more frequently than in the raspberry. A single blackberry leaf may contain all the leaflet patterns shown in figure 2 and in raspberry several types may be expressed in a single leaf.

The infected areas of young leaves of both blackberry and raspberry are at first light green, later become yellow, and as the leaves reach maturity, these infected areas become totally white. Blodgett[3] found that there is a similar color sequence of the chlorotic areas in peach leaves infected with peach calico.

Leaf tissue from chlorotic areas was examined for inclusion bodies

[3] Blodgett, Earle C. Peach calico. Phytopath. **34**: 650–657. 1944.

using the trypan-blue technique described by McWhorter,[4] but none were observed.

Transmission by grafting in over 50 per cent of the attempts together with the type of symptoms produced indicate that the causal agent is a virus. A search of the literature has not revealed any reference to such a virus disease in blackberry or raspberry.

No economic importance has been attached to this new virus in blackberry, but since it occurs in the raspberry growing area of Maryland, and since black raspberry is a host of the virus, it may be of potential importance.

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MORPHOLOGY AND TAXONOMY OF THE ONION PINK-ROOT FUNGUS[1]

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INTRODUCTION

Pink root of onion (*Allium cepa* L.) is incited by a soil-inhabiting fungus which has become endemic in many onion-growing sections of the United States. The causal organism was thought to be a species of *Fusarium* by Taubenhaus and Mally (10), who named it *Fusarium malli* Taub. They based their conclusions on studies of disease development of onion sets planted in previously steam-sterilized, Fusarium-inoculated soil. Five to 15 per cent of the plants in the inoculated soil became infected as compared with 100 per cent in the non-sterilized soil. They reasoned that this difference in infection was probably due to other organisms, especially other species of *Fusarium*, acting in combination with *Fusarium malli*. Sideris (8) named several new species of *Fusarium* which he regarded as capable of inciting the disease but only a small percentage of the plants in his inoculation experiments became infected. In inoculation experiments with various Fusaria, including authentic cultures from Sideris, Hansen (3) failed to produce the disease. He secured positive results from inoculation with a fungus (*Phoma terrestris* Hansen) isolated from diseased roots. With this fungus he obtained production of pycnidia in culture but since only pycnidial primordia were found in infected roots it was concluded that mature pycnidia apparently never occurred on infected onion roots. The pycnidia were described as subglobose, ostiolate, papillate, dark brown to black, carbonaceous, 170 to 350 μ in size, and single or gregarious. The mature pycnidia varied considerably not only in size, but also in shape, papillation, and length and number of ostioles. Some isolates were predominantly pycnidial and others were mostly or entirely mycelial. There was a tendency of isolates to produce pycnidia abundantly when transfers were made with spores and to become mycelial if transfers were made with mycelium. In a later paper (4) Hansen referred to this tendency as "the dual phenomenon." He considered that the changes which occurred in this fungus in culture, as in various other members of Fungi Imperfecti, were not due to mutation but to the development of thalli which had different complements of nuclei. Thus within *Phoma terrestris*, which he reported to have binucleate spores, he separated out three different types by isolating single-spore lines from the original cul-

[1] This investigation was carried on under cooperative agreement between the Department of Plant Pathology, University of Wisconsin, and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

[2] The writers wish to express their appreciation to Mr. Eugene Herrling for preparation of the illustrations.

ture. These were designated as mycelial (M), mycelial-conidial (MC), and conidial (C) types. The M and MC types produced normal pycnidia whereas the C type produced pycnidia with beaks frequently several times as long as the diameter of the pycnidium.

In the present investigation a large number of isolates from various sections of United States were assembled. A morphological study was made of these cultures and the range of morphological variability was determined.

METHODS AND MATERIALS

Most of the isolates of the pink-root organism used in the course of this work were made directly from infected roots. The infected material arrived in excellent condition for making direct isolations when it was packed in moist cotton or in moist soil. In other cases, isolations were made from plants grown on samples of infested soil. A culture of the fungus was obtained from Michigan and one from California.

Recently infected, firm, pink roots were found to be the most desirable for isolation, but success was also had with dry material. The roots were first washed thoroughly in distilled water, then immersed in 1–1000 mercuric chloride solution for three to five minutes or from five minutes to three hours in BK solution made by adding one-half teaspoonful of B-K powder (50 per cent calcium hypochlorite) to 20 cc. water. The roots immersed in mercuric chloride were washed in sterile water and plated; those in B-K solution were removed directly to agar plates.

A wide variety of nutrient media was used with equal success, each plate being acidified with one drop of 75 per cent lactic acid. Thirty plates or more were used for each lot of diseased material. Colonies appeared from the plated roots in three to eight days, depending on the medium and the method of surface sterilization. Pure cultures of the organism were often obtained when dead, diseased roots, containing numerous pycnidial primordia when dug from the soil, were surface sterilized with B-K solution for three hours and plated. In most cases other fungi appeared from the roots in close association with the pink-root organism. The colonies of the latter could be recognized by their relatively slow, compact growth, by the greyish aerial mycelium, and especially by the production of pigment, which varied in color with the type and reaction of the medium but was usually a shade of red. Large numbers of transfers were made from the leading edges of such colonies. The sources of the isolates, their relative virulence and their sporulating capacities are given in table 1.

EXPERIMENTAL RESULTS

Variability of the Isolates

When the isolates were carried on culture media in test tubes and mycelial transfers made about every four months, most of them became mycelial in type. Some of them were kept in MC condition by using the

original bit of inoculum in each successive transfer. No mycelial line, *e.g.*, the hyphal-tip lines of the Colorado, Utah, Louisiana, and Texas isolates, ever reverted to the pycnidial type. The Utah isolate was carried in culture for six years and in this interval never produced pycnidia either in culture or on the host. However, it often produced dark-colored stromata in culture and on the host.

The use of mycelium in transferring did not necessarily result in a loss of the tendency to produce pycnidia abundantly. A pycnidial isolate from Louisiana retained the sporulating character for 18 months when it was kept growing on Petri dishes and transfers were made with mycelium from the leading edge of the colony. There appeared to be a relation between aging and the change from the pycnidial to the mycelial type as mycelial transfers from old plates of pycnidial cultures resulted in my-

TABLE 1.—*The sources of the isolates of the onion pink-root organism, their virulence and their sporulating capacities*

Source of isolate	Virulence	Pycnidial production	
		In culture	On the host
Louisiana	+++[a]	+[b]	+[b]
California	+++	+	+
Colorado	+++	+	+
Texas	+++	+	+
Utah	++	−	−
Illinois	++	+	+
Iowa	++	+	+
Massachusetts	+	−	−
New York	+	+	+
Wisconsin	+	+	−
Michigan	+	−	−

[a] +++ = highly virulent; ++ = moderately virulent; + = mildly virulent.
[b] + = pycnidia produced; − = no formation of pycnidia.

celial cultures or gave mycelial sectors. A similar phenomenon was reported by Hansen and Snyder (5) for *Penicillium notatum* Westling.

In attempts to induce reversion of the mycelial type to the pycnidial type, cultures were made on a variety of natural and synthetic media; tubes were placed out of doors; and cultures on a variety of media were exposed to ultra-violet light for a range of time intervals. In no case was a reversion from a true mycelial type to a sporulating type observed.

In one experiment, seed was planted in sand infested 30 days previously with a pycnidial isolate. Severe infection of the seedlings resulted but pycnidia were very sparse. However, seedlings grown previously in the same sand had borne abundant pycnidia. In another case sand was infested with a mixture of equal parts of inoculum from a pycnidial and a mycelial strain. Pycnidial production on the seedlings grown in the infested sand was sparse although abundant pycnidia were produced on seedlings in sand infested with the pycnidial isolate only.

Twelve isolates in 13-months-old dehydrated cultures were all viable when transferred to fresh medium.

Morphology of the Pycnidium

The pycnidia produced by the sporulating isolates conformed to the description by Hansen of *Phoma terrestris* with the exception of one character. In this investigation the pycnidia were found to be setose. Hansen did not state that the pycnidium was smooth and he did not include a

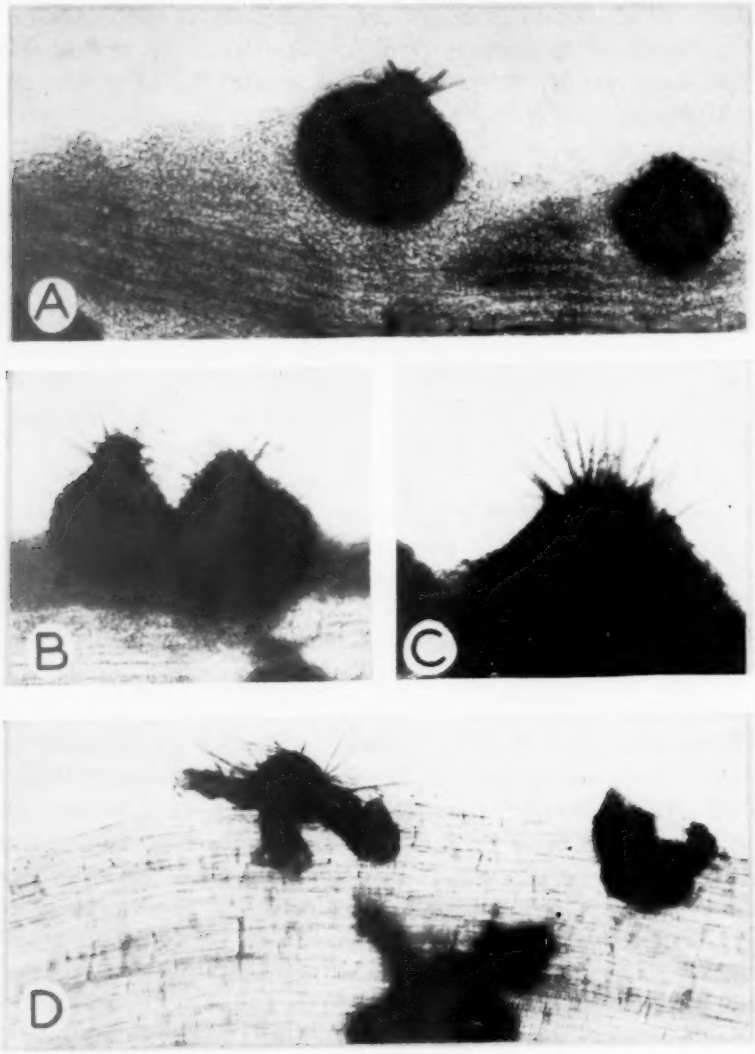

FIG. 1. Pycnidia produced on onion roots. ×99. A. The California isolate on root of onion seedling grown in inoculated sand. The pycnidia have short setae and are relatively deep-seated in the root tissue. B and C. The Louisiana isolate on root of onion seedling grown in inoculated sand. The pycnidia have long setae and are not so deep-seated as in the case of the California isolate. D. The Louisiana isolate, after three single-spore transfers, on root from a bulb grown in infested soil. The deformed and slightly smaller pycnidia are typical of those in pure culture after repeated single-spore transfers.

drawing or photomicrograph of a pycnidium. Carvajal (1) and Sprague (9) secured pycnidia in the cultures of some isolates but they did not describe or illustrate them.

The length, number and position of the setae on the pycnidium varied considerably among the different isolates. The setae of the California isolate (Fig. 1, A) were short, few in number, and grouped around the ostiole of the pycnidium. Those of the Louisiana isolate were longer and more numerous. They were scattered over the surface of the pycnidium but were most numerous about the ostiole (Fig. 1, B and C). The setae of the New York (Fig. 2, B), Illinois, Iowa, Wisconsin, and Colorado isolates were medium to long, few to numerous, and grouped almost exclusively about the ostiole.

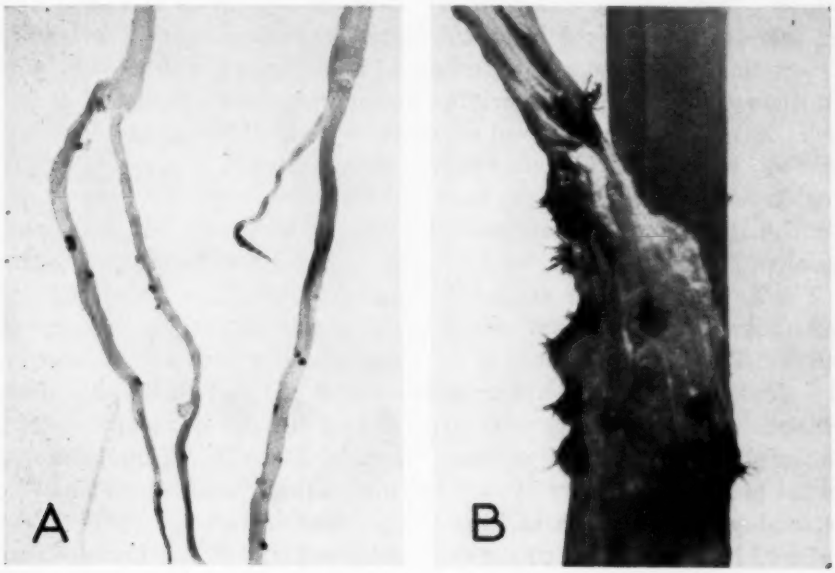

FIG. 2. Pycnidia produced on onion seedlings grown in sand culture. A. The Louisiana isolate on roots. ×5. B. The New York isolate on a dead cotyledon at the sand surface. ×49. The setae are concentrated around the ostiole.

The pycnidia also varied considerably in size, shape, and papillation. Those of the Louisiana isolate (Fig. 1, B) were typically globose and slightly beaked. Those of the other isolates tended to be subglobose and papillate, especially when produced on the host. An occasional pycnidium was found with two or even three ostioles. More variability was encountered in pycnidia produced in culture than in pycnidia produced on the host. After two successive single-spore transfers, the Louisiana isolate produced pycnidia which were less setose, more irregular in shape and smaller in size than those of the original isolate. The same type of pycnidium was produced in culture and on the host (Fig. 1, D).

Pycnidial Production on the Host

Pycnidia of the pink-root organism have not been reported previously on the host. This indicates that they are produced rarely on the plant in the field. Microscopical examination of diseased roots from the Racine, Wisconsin, area showed numerous pycnidial primordia varying in size but smaller than the mature pycnidium. Pycnidia were produced in cultures of isolates from these roots.

Mature pycnidia were first found in June, 1946, on the roots of onion seedlings grown in sand which had been infested with the first isolate secured from Louisiana specimens. This isolate also produced pycnidia in culture. Two successive single-spore transfers were made and mass transfers therefrom were used in all subsequent experiments with that isolate. A short time later pycnidia were found on seedlings grown in sand mixed with the roots and soil of the original consignment of specimens from Louisiana. Pycnidia were produced consistently on the host in sand-culture experiments in which this isolate was used as inoculum (Fig. 2, A). Moreover, pycnidia were produced on the roots in all cases in subsequent experiments in which the hydrogen-ion concentration of the host nutrient, salt concentration of the host nutrient, sand temperature, and host variety were varied.

The New York isolate produced numerous pycnidia on the dead cotyledons (Fig. 2, B) at the surface of the substrate in the sand-temperature studies. They were produced at all temperatures except 16° C. where the cotyledons of the seedlings were still alive at the end of the experiment. In another series pycnidia were produced on the dead cotyledons and on dead seedlings at the sand surface. Certain Texas, Iowa, and California isolates produced pycnidia in sand culture on dead plants above and below the sand surface, each in at least six different instances. Pycnidia were produced in sand culture on the dead outer scales of White Portugal bulbs inoculated with the Louisiana and California isolates. With soil culture, pycnidia were produced on the roots only by the Louisiana isolate (Fig. 1, D.).

Histological examination of diseased roots revealed the presence of pycnidial primordia in the cortical region of the root. Immature pycnidia were also found that were completely covered by the epidermis of the root or cotyledon. Serial sections of mature pycnidia on the roots revealed that in sections through the side of the pycnidium the latter was completely covered by the host epidermis while in sections through the central portion of the pycnidium the latter protruded through the host epidermis (Fig. 3).

Taxonomy of the Fungus

The history of *Phoma* as given by Shear (7) reveals the taxonomic confusion that exists with this genus. It was set up by Fries in 1819 and was completely emended by Desmazieres in 1849. Saccardo's emended descrip-

tion of the genus in 1882 was as follows: perithecia smooth, without a beak, subcutaneous, not maculicolous, and spores without appendages. The characteristics of the genus as given by Grove (2, p. 58) are as follows:

> "PHOMA. Pycnidia covered by the epidermis or the periderm, then erumpent, membranaceous or subcoriaceous, occasionally carbonaceous, globose or depressed, glabrous, without a distinct or prolonged beak, but with a small (sometimes indistinct) ostiole which may be simply impressed, but is more often papillate. Spores, oblong or ovoid, more rarely fusoid, or subcylindrical, seldom globose, continuous, hyaline, typically biguttulate; sporophores simple, sometimes very short or imperceptible."

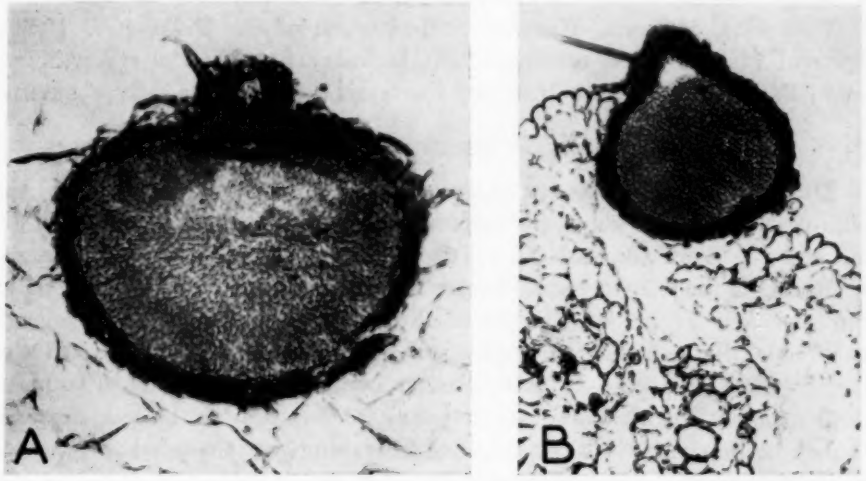

FIG. 3. Cross-sections of pycnidia and host tissue from onion seedlings grown in inoculated sand. A. California isolate on cotyledon. ×260. B. Louisiana isolate on root. ×180.

The genus *Pyrenochaeta* was set up by de Notaris about 1845. His original description was not available but the one given by Grove (2) follows:

> "PYRENOCHAETA. Pycnidia globose or conical, immersed, then erumpent, membranaceous or subcarbonaceous, pierced at the apex, beset with stiff bristles, especially toward the top; texture parenchymatous or prosenchymatous. Spores 1-celled, of various shapes, hyaline or faintly coloured; sporophores slender."
>
> "The characteristic of this genus lies in the possession of bristles clothing the external surface of the pycnidium; these are not so thick and stiff as those of Vermicularia."

The genus *Phoma* can no longer apply to the causal organism of pink root of onion because the genus is characterized as having a smooth pycnidium. In view of the fact that in each of the isolates in which pycnidia have been observed the latter have been setose, the species is trans-

ferred to the genus *Pyrenochaeta* and is designated as **Pyrenochaeta terrestris** (Hansen) nov. comb.

Morphology.—Pycnidia globose to subglobose, immersed, then erumpent, ostiolate, papillate to slightly beaked, dark brown to black, subcarbonaceous, 120–450 μ, slightly to definitely setose when mature, may occur singly, frequently gregarious. Setae light to dark brown, 1–5 septate, 8–120 μ in length, few to numerous, generally around the ostiole but may occur over the entire pycnidium. Conidia continuous, hyaline, oblong-ovoid, 3.7–5.8 × 1.8–2.4 μ, biguttulate, sessile in the pycnidium, escaping as a gelatinous mass through ruptures or as a cirrus through the ostiole. Mycelium septate, hyaline, guttulate.

Specimens are deposited at the following: Herbarium of the University of Wisconsin, Madison, Wisconsin; Herbarium of the Division of Mycology and Plant Disease Survey, Plant Industry Station, Beltsville, Maryland; Farlow Herbarium, Harvard University, Cambridge, Massachusetts.

DISCUSSION

Differences in morphology of the pycnidia of the various isolates of the pink-root organisms were very pronounced, especially between the California and the Louisiana isolates. Since all isolates caused the same disease symptoms it was considered advisable that all the strains be included under *Pyrenochaeta terrestris*. The emended description was made broad enough to include all the strains studied. It is interesting to speculate as to why the setae have not been noticed by other investigators. It should be noted that in the California isolate the setose characteristic was the least marked. It may be that Hansen's (3) original description was made on the basis of a similar isolate. Another factor was that the pycnidia produced in culture, especially on a medium high in dextrose, were overgrown with mycelium and the setose character was easily missed, especially if the characteristic was not marked. It was when pycnidia were found produced on the host that the setae attracted attention.

The pycnidia have never been found on the host in the field. Experiments with the Louisiana pycnidial isolate showed that pycnidia were produced on roots in unsterilized, artificially infested soil. However, none of the other isolates produced pycnidia on the host plant in soil even though some of them did so on seedlings in sand culture when the same inoculum was used. Inoculation experiments in sand and in soil with pycnidial and mycelial strains have shown that a pycnidial isolate must be used as inoculum before pycnidia are produced on the host. Other investigators have probably inoculated with mycelial isolates, or with inoculum from 20- to 45-day cultures of pycnidial strains which tend to become mycelial in character with age. In this investigation 10-day inoculum was used in all experiments. Many of the original isolations were mycelial in type. If they were pycnidal in type, special efforts, such as spore transfers or fre-

quent mycelial transfers, had to be made to keep them from becoming mycelial. Kreutzer (6) made extensive inoculation experiments, but only one of his isolates produced pycnidia in culture and thus only one pycnidial line was available to him.

A mycelial isolate was never found to revert to the pycnidial type and all attempts at artificial induction of pycnidia failed. The change from the pycnidial type to the mycelial type occurred very frequently when mycelial transfers were made from old pycnidial cultures. However, if transfers of young cultures were made continuously the line could be kept sporulating, probably indefinitely, by the use of mycelial transfers. The use of a mycelial transfer in itself did not cause a change from the pycnidial to the mycelial type. Pycnidial cultures tended to become overgrown with the mycelial type after they were 20 days old. This indicated a connection between aging of the culture and mutation to the mycelial type, a phenomenon noted in other fungi.

SUMMARY

A large number of isolates of the pink-root organism from various sections of the United States were assembled and their morphology was studied. In some of the isolates, pycnidia were produced in culture media and with every such isolate they were produced either on roots or cotyledons of plants grown in inoculated, white silica sand. In the case of only one isolate were pycnidia produced on the host grown in soil. This is the first report of pycnidia on the host, but as yet they have not been found in nature.

The pycnidia were found to be setose in all cases, although there was considerable variation between isolates in number and distribution of setae and in other morphological characteristics of the pycnidium. Because of the setose character of the pycnidium the species was transferred to the genus *Pyrenochaeta*.

Isolates which readily produced pycnidia in culture lost this character when transfers were made with mycelium from old cultures, in which there was a tendency for the fungus to mutate to the mycelial type. When sporulating cultures were transferred with mycelium from the advancing edge of the colony, or with spores, the sporulating character was retained. No production of sporulating mutants in mycelial lines was observed.

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APHID TRANSMISSION OF LILY VIRUSES DURING STORAGE OF THE BULBS

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Virus diseases constitute a major hazard in commercial cultivation of lilies. In the culture of Easter lily (*Lilium longiflorum* Thunb.) necrotic fleck and rosette diseases[3] must be avoided, preferably by selecting planting stock that is free from these diseases and planting it at a distance from known sources. Some garden lilies, notably *L. formosanum* Stapf and *L. tigrinum* Ker-Gawl., must also be kept free from the mottle virus[4] that is common in commercial stocks of Easter lily and Madonna lily (*L. candidum* L.) but is not obviously damaging in these species. Some growers therefore produce *L. formosanum* and other seed-propagated species in separate fields from the self-sterile and hybrid forms which must be propagated from bulbs. The usual practice, however, is to assemble all lily-bulb stocks in one storehouse for cleaning, grading, and packaging. Vector species of aphids may transmit viruses from sprout to sprout during this interval in the storehouse, and may thus vitiate the benefit from isolation provided by culture of the lily stocks in different fields.

Our attention was drawn to this problem by an Oregon grower who had produced virus-free *Lilium formosanum* under suitable isolation for several years, but later found an outbreak of lily mottle virus in the planting. No source of this virus could be detected near the field, but it was reported that the *L. formosanum* bulbs had been cleaned and graded in the same storehouse with *L. candidum* bulbs that had been grown elsewhere.

Experiments were therefore planned to determine whether *Aphis gossypii* Glover and *Myzus persicae* Sulz. would colonize on lily bulbs and sprouts during storage, and whether *A. gossypii* could transmit from sprout to sprout the persistent lily rosette virus, and *M. persicae* could transmit the nonpersistent lily mottle and cucumber mosaic viruses.

Lily mottle virus was taken from naturally infected stock of *Lilium longiflorum* var. Creole originally from Florida. The virus from this source material is similar to and possibly identical with that common in *L. candidum*. Cucumber mosaic virus, a strain originally isolated from necrotic-flecked stock of *L. longiflorum* var. Harrisi from Bermuda, was maintained in seedling Easter lilies. Lily rosette virus, originally obtained from Florida Easter lilies, was also cultured in Easter lily seedlings. Seedlings of

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[3] Brierley, Philip, and Floyd F. Smith. Studies on lily virus diseases: the necrotic-fleck complex in *Lilium longiflorum*. Phytopath. **34**: 529–555. 1944.

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L. formosanum or *L. longiflorum* served as test plants for lily mottle virus and for lily rosette virus. As test plants for cucumber mosaic a stock of Creole lilies was selected that was free from necrotic fleck but which responded with fleck symptoms when infected with cucumber mosaic.[3] Potted plants in good vegetative growth or bulbs out of cool, dark storage with colorless shoot or sprout growth ½ to 2 inches long were used to supply the virus and as test plants.

TABLE 1.—*Transmission Trials with Lily Mottle Virus, Cucumber Mosaic Virus, and Lily Rosette Virus by Aphis gossypii, Myzus circumflexus, and M. persicae*

Aphid species	Source of virus	Plants inoculated	Ratio of plants infected to plants exposed
		Lily Mottle Virus	
Myzus circumflexus	Sprouts of *Lilium longiflorum* var. Creole	Sprouts of seedling *L. longiflorum*	0/5
Myzus persicae	Do	Do	3/18
		Growing plants of *L. longiflorum*	2/5
		Growing plants of *L. formosanum*	10/10
		Bulb scales of *L. formosanum*	0/9
	Bulb scales of *L. longiflorum* var. Creole	Growing plants of *L. formosanum*	1/10
		Cucumber Mosaic Virus	
Myzus persicae	Growing plant of *L. longiflorum* seedling	Sprouts of *L. longiflorum* var. Creole	2/2
		Growing plants of *L. longiflorum* var. Creole	4/5
		Lily Rosette Virus	
Aphis gossypii	Sprouts of *L. longiflorum* seedling	Growing plants of *L. longiflorum*	3/5
		Sprouts of *L. longiflorum*	7/7
		Sprouts of *L. longiflorum* var. Creole	2/3

Myzus persicae, known from previous studies to be an efficient vector of lily mottle virus and of cucumber mosaic virus, was used in tests with these viruses. This species was cultured on cabbage (*Brassica oleracea* var. *capitata* L.), which is nonsusceptible to these viruses, and the aphids were starved for 2 hours before they were permitted to feed on source plants—a procedure known to improve efficiency of transfer of nonpersistent viruses. *M. persicae* fed readily on the leaf scales of sprouts of source bulbs after such a period of starvation. The aphids were placed on the sprouts of test bulbs and remained there for 2 days, but when moved to the green leaves of growing lilies all but a few individuals wandered off.

Myzus circumflexus Buckt., used in one test with lily mottle virus, was reared on potato (*Solanum tuberosum* L.) and starved for 3 hours before being placed on sprouts of the source bulbs. This species fed readily on the sprouts of stored lily bulbs. *Aphis gossypii,* reared on chrysanthemum (*Chrysanthemum hortorum* Bailey), fed readily and increased to dense colonies on sprouts of stored Easter lily bulbs that served as sources of lily rosette virus. *A. gossypii* was permitted to feed for 6 days on this source material to allow it to acquire this persistent virus, before it was moved to sprouts or to growing plants of test lilies.

Mechanical inoculations from sprouted bulbs of Creole lilies affected with lily mottle virus were made on growing plants of *Lilium formosanum.* The Creole bulbs had been held in dry peat for 5½ months at 50° F., and showed white shoot growth ½ to 1 inch long but no root growth. Bulb scales were flaccid after long storage. The white shoot tips of 7 of these bulbs were ground in a mortar with a little water, and the macerated tissue was rubbed over 10 young *L. formosanum* plants. Three of these plants showed typical lily mottle symptoms. Symptoms were noted 10 days after exposure and confirmed after 30 days. A transfer of *Myzus persicae* (Table 1) from comparable material yielded 10 infections in 10 plants.

In a second test bulb scales of the same bulbs served as source of the virus. The outer, shriveled scales were discarded, and intermediate scales that were less flaccid were chosen. When ground with a little water they produced 8 infections in 10 inoculated plants, the symptoms and time interval corresponding to those in the first test. Another transfer of *Myzus persicae* from comparable material yielded 1 infection in 10 inoculated plants. These results show that the lily mottle virus is present in both scales and shoots of stored Easter lilies. They also imply that dormant lily bulbs might be indexed to determine the presence of this virus, but as this point has not been studied further, the efficiency of such indexing remains unknown.

The data in table 1 show that *Myzus persicae* can acquire the lily mottle virus from the colorless sprouts and the bulb scales of stored Easter lily bulbs. This aphid can transmit the virus to similar sprouts of Easter lily seedlings and to growing plants of this species or of *Lilium formosanum.* In a single trial, however, the aphid failed to introduce this virus into the bulb scales of *L. formosanum*—an indication that bulbs that have not begun to sprout in storage are less likely to become infected. *M. circumflexus* did not transmit lily mottle virus from sprout to sprout in storage. This species has also failed consistently to transmit this virus from plant to plant.[5]

Cucumber mosaic virus was readily transmitted by *Myzus persicae* from growing plants of Easter lily to growing plants of Creole lilies, or to the sprouts of these lilies in storage. Typical symptoms of necrotic fleck were induced in the Creole lilies in both tests.

Lily rosette virus was readily transmitted from sprout to sprout or from sprout to growing plants of Easter lilies by *Aphis gossypii.* It appears that

[5] See footnote 4.

this species can transmit this virus during storage, and can carry it to growing plants in nearby fields. As *A. gossypii* colonizes readily on the shoots of stored bulbs, it also seems likely that this insect may be transported on them. In addition to carrying lily rosette virus, this species is a known carrier of lily mottle, lily symptomless, and cucumber mosaic viruses.[6]

It is apparent that lily viruses of both persistent and nonpersistent classes may be transmitted during storage of the bulbs by appropriate aphid vectors. Evidence is shown (Table 1) that one lily virus can be carried from growing plants to sprouting bulbs, and that two others can be carried from sprouting bulbs to growing plants. It seems likely, therefore, that lily viruses can be carried from the field to the storehouse and from the storehouse to the field when suitable aphid species are present. The hazard of long-distance spread by the flight of aphids is of course greater with the persistent viruses. In planning segregation of tolerant and intolerant lily species, it is advisable to give consideration to the relation of the grading and storage facilities to the field plantings, and to keep aphids out of the storage house.

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[6] See footnotes 3 and 4.

THE INEFFICACY OF ETHYLENE CHLOROBROMIDE AS A THERAPEUTIC AGENT IN THE TREATMENT OF GARDENIAS INFECTED WITH THE ROOT-KNOT NEMATODE[1]

A. C. Tarjan

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Tests were conducted at Beltsville, Maryland, in which 30 two-year-old cuttings of *Gardenia jasminoides* Ellis with roots badly infected with the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, were subjected to various concentrations of ethylene chlorobromide.[2] The plants were divided into two groups, the first receiving only one application of the chemical, and the second receiving two applications of the chemical two weeks apart. The second application on the second group was planned for the following reason:—if the chemical was lethal to all living nematodes but failed to destroy the eggs, a two-week period after the first application would allow for the hatching of most of the eggs and the resulting nematode larvae would then be vulnerable to the toxic effects of the second application.

Plants which had been growing in six-inch pots were transplanted to eight-inch pots so that there would be a one-inch space, around the perimeter of the pots, which contained no roots. They were then watered and left for two days in order that moisture would be uniform throughout the soil. Since the maximum amount of chemical the plants would withstand had been roughly determined, in a previous experiment, to be below 0.50 ml., treatments applied were 0.40 ml., 0.25 ml., 0.10 ml., and 0.05 ml. of ethylene chlorobromide. Because of the small amounts of the chemical involved, distilled water emulsions were prepared so that each plant received a total of 8 ml. of the emulsion. Before application, the emulsion was thoroughly agitated in order that each replicate would receive the same percentage of ethylene chlorobromide in proportion to distilled water.

At the time of application, soil moisture was determined to be 7.01 per cent by weight while the soil temperature was 67° F. Method of application was as follows: four mutually equidistant holes were pierced two inches deep into the soil around the perimeter within each of the eight-inch pots containing the plants. Accuracy of dosage was attained by use of a graduated hypodermic syringe which injected four equal aliquots of the total amount into the holes in the soil of each pot; the holes were then sealed with soil. In this manner, none of the emulsion was poured directly on the roots since the perimeter of the root ball was located one inch from the perimeter of the pot. After treatment, all pots were watered lightly.

[1] Scientific Article No. A195. Contribution No. 2108 of the Maryland Agricultural Experiment Station (Division of Plant Pathology).

The author offers his sincere appreciation to the Bartlett Tree Expert Company, Stamford, Conn., for financial assistance rendered in making this work possible.

[2] 1-bromo-2-chloroethane (CH_2ClCH_2Br). Experimental sample was supplied by Richard P. Porter of Innis, Speiden and Co., New York.

The second group received an additional treatment of 0.05 ml., 0.10 ml., and 0.25 ml. of the chemical in emulsion form after two weeks had elapsed; there was no additional application of the 0.40-ml. treatment since one application proved phytotoxic. Each group was harvested one month after its final treatment.

Preliminary inspections of a few galls after treatment revealed the presence of active nematodes. In an effort to obtain some type of tabular data that would illustrate the degree of efficacy of ethylene chlorobromide, quantitative determinations of parasite population in the roots were obtained by a modification of the Baermann technique—a method proven valid for obtaining root nematode population counts in studies of the meadow nematode. This method essentially consists of thoroughly washing the soil away from the roots of each plant, drying the root surfaces with filter paper, and then cutting them up finely until 5 gm. of root clippings are obtained.

TABLE 1.—*The effect of ethylene chlorobromide on root-knot nematode populations in Gardenia jasminoides roots*

Treatment	Root population counts					
	One application			Two applications		
	Replicates		Mean	Replicates		Mean
Control	30, 68, 79[a]		59	40, 45, 69		51
0.05 ml.	46, 80, 195		107	36, 41, 68		48
0.10 ml.	77, 109, 272		153	70, 108, 365		181
0.25 ml.	25, 52, —[b]		39	—, —, —		—
0.40 ml.	—, —, —		—	—, —, —		—

[a] Each number represents the mean of nematode counts obtained from three 5-ml. samples.

[b] Plants died as a result of treatment.

This is put in the Baermann apparatus[3] which consists of a six-inch glass funnel in which a square piece of finely meshed cheesecloth is suspended by means of clamps. A short piece of rubber tubing attached to the end of the funnel is closed by means of a metal clamp. The root sample is put into the pocket formed by the suspended cheesecloth, and water is poured in until the root clippings are completely submerged. This apparatus is effective in that the nematodes evacuating the root fall to the cheesecloth, wriggle through the meshes, and fall to the neck of the funnel. In adopting this method for material infested with root-knot nematode, it should be pointed out that only the larval forms are capable of leaving the roots, consequently this method is in reality "an assay of the potency of the infection"[4] represented by the diseased root samples.

After allowing the root samples to remain in the funnels for 1½ days, a ten-ml. sample was drawn off from each funnel. This was increased in

[3] Filipjev, I. N., and J. H. Shuurmans Stekhoven, Jr. A Manual of Agricultural Helminthology. 878 pp. Leiden, Holland: E. J. Brill. 1941.

[4] As suggested by Dr. G. Steiner, Chief of the Division of Nematology, Bureau of Plant Industry, U.S.D.A., under whose supervision this work was carried out.

volume to 100 ml. by the addition of water, and, after thorough agitation, the nematodes in each of the three aliquots of five ml. were counted. The mean of these counts was then recorded.

With hopes of getting some sort of correlation between population counts and root condition previous to sampling, each root system was rated from "1" to "10," "1" signifying healthy roots and "10" signifying dead roots. No correlation, however, was apparent in the final analysis.

As shown in table 1, one application of 0.25 ml. and two applications of 0.05 ml. resulted in means lower than the controls. Although the results of the former treatment are explainable, no sound reason can be offered explaining why the 0.05 ml. treatment mean is less than the 0.10 ml. treatment mean within the two-application group. Another enigma is apparent in the lower mean of the controls in contrast to the 0.05 ml. and 0.10 ml. treatment means of the one application group and to the 0.10 ml. treatment mean in the two application group.

The explanation of these results may be: (1) The sampling method, as used in this experiment, may not have been reliable; (2) The fumigant may have weakened the resistance of the plant, thus increasing its susceptibility to penetration and subsequent infection by the root-knot nematode; (3) The fumigant may have killed the natural enemies (predatory nematodes and parasitic fungi) of the nematodes involved; or (4) There may have been a stimulating effect on the development of the parasites by the chemical treatments. Statistical analysis proved the results were not significant because of the wide variation of population counts within replicates.

The only figure which might illustrate a nematocidal tendency on the part of the soil fumigant were the results obtained using a treatment of 0.25 ml. in the one-application group. Since this treatment killed one of the replicates, its deleterious effects are not compensated for by the reduction of root nematode populations. Therefore, within the limits of this experiment, ethylene chlorobromide is not a suitable therapeutic agent for root-knot infested Gardenias.

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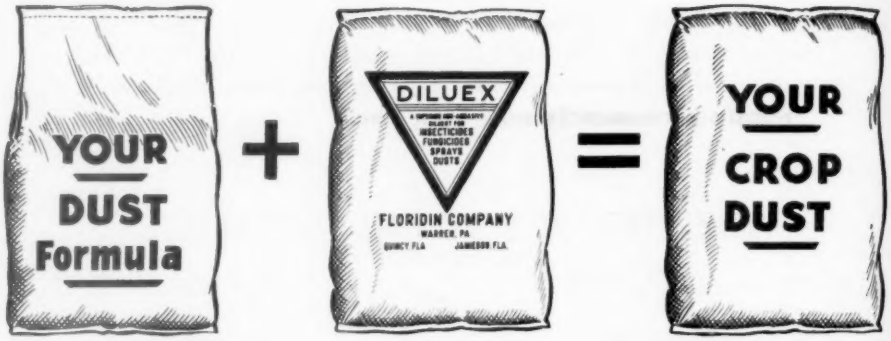

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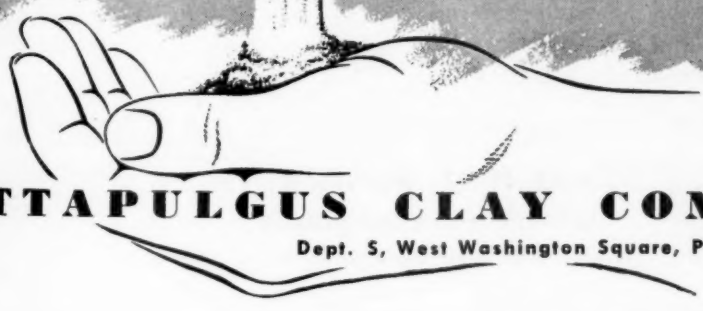

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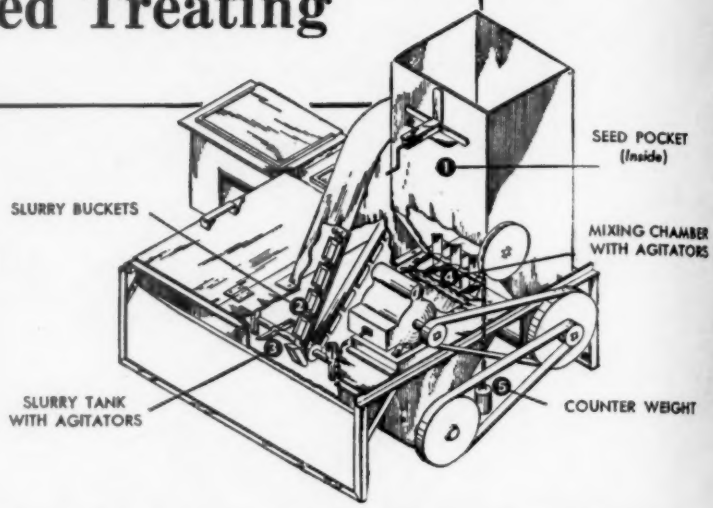

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